HONEY

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I. INTRODUCTION

Honey is the only sweetening material that can be stored and used exactly as produced in nature. No refining or processing is necessary before enjoying this unique material, which can be traced through the entire span of recorded history. Honey, which was man's first sweet, was used earliest as a ceremonial material and a medicinal ingredient. Not until the era of the Greeks and Romans did honey come to be regarded as a food also. It so remained until relatively recently displaced by cane and beet sugar during the past 100 years.

Honey is the sweet, viscous substance elaborated by the honeybee from the nectar of plants. This simple definition excludes honeydew honey, which is produced by the bee from honeydew excreted by various plant-sucking insects. The bee harvests, transports, and processes the nectar to honey, and packages and stores it in the comb. Processing consists of simultaneously reducing the moisture content from the 30–60% common to nectars to the self-preserving range of 15–19%, inverting the considerable proportion of sucrose by the addition of invertase, preserving it meanwhile by adding a glucose oxidase which produces small amounts of acidity and hydrogen peroxide. Ripening takes place in open cells of the comb, which are sealed when the honey reaches full density. The combs, of course, are constructed by the bees from wax they secrete, the production of which requires about 8–10 times its weight in honey. As a unique natural product, honey produces an interesting link to earlier times, and a wealth of observations such as: a bee colony flies about 75,000 miles (121,000 km) to produce a pound (454 gm) of honey, but the "fuel" consumption in this (at 1 million miles per gallon or 426,000 km per liter) is only about three ounces (85 gm).

The purpose of this review is to provide scientists and technologists with information on the composition, properties, processing, and uses of honey necessary to making informed decisions about its use and value in their operations.

II. PRODUCTION AND PROCESSING

A. PRINCIPAL AREAS AND TYPES

Honey, even when processed for commercial use, is essentially a natural product. As produced, it is highly variable, particularly in color, flavor, moisture content, and sugar composition, indeed in nearly every constituent. These attributes depend upon climate, the floral type, and individual beekeeping practices. While bees are kept in all 50 states of the United States and in every country of the world, conditions favorable to commercial beekeeping (honey production) are not as widely available. Further, as agricultural practices and crops change, the value of areas for beekeeping or the quality, type, and amount of honey produced will be influenced. Table I shows honey production, imports, and exports for the major honey-producing and consuming countries, providing a summary of world trade in the commodity.*

1. United States

About one-third of the United States honey crop is sold by the producer directly to the consumer, the remainder to packers. Nearly half of the crop is produced by about 1200 fulltime beekeepers (400 or more hives), about two-fifths by parttime beekeepers (25–400 colonies), and the remainder by hobbyists (<25 colonies) (Bauer, 1960). Each of the 48 contiguous states produces at least a million pounds (454,000 kg) annually, except Delaware, Maryland, South Carolina, those in New England, and the largely desert states of Nevada and New Mexico; the primary producing areas are the intermountain area, west coast, central states, Texas, and the southeast. California and Florida vie for the greatest production, averaging 20–30 million pounds (9.07–13.61 million kg) annually. Minnesota, South Dakota, and Texas generally exceed 10 million pounds (4.54 million kg). Annual variation in a state's output of 50–100% is not uncommon.

Honey is characterized by its color and floral type. A limited number of commercially available honeys are essentially monofloral, i.e., not appreciably blended by the bees. This includes such types as clover, alfalfa, tupelo, "orange" (which is actually better called "citrus" since grapefruit is present). gallberry, and cotton. "Clover" honey may be relatively pure, or may be called "clover" but be more or less naturally blended with other sources in areas where clover pasturage is not widespread. This is generally obvious because of the darker color. A group of naturally blended honeys is available on a consistent

*More detailed statistics are available annually from the Foreign Agricultural Service, U.S. Department of Agriculture, Washington, D.C. 20250.

TABLE I

HONEY PRODUCTION AND TRADE[a,b]

(in millions of pounds)[c,d]

Country	Production	Imports	Exports
United States	212.4	25.2	8.7
Canada	50.3	0.9	13.2
Mexico	80.3		57.7
Cuba	10.1		6.0
El Salvador	3.3		3.3
Guatemala	6.8		5.9
Argentina	51.8		38.1
Brazil	11.4		2.5
Chile	15.0		2.0
Austria	13.5	7.6	
Belgium-Luxembourg	2.2	5.5	
France	22.1	9.9	2.4
West Germany	27.2	97.9	3.4
Greece	19.8		2.7
Italy	13.6	3.5	
Spain	20.6		18.2
Netherlands	0.4	6.6	1.2
Switzerland	4.2	10.1	
United Kingdom	8.1	32.5	
Bulgaria	13.7		6.1
Czechoslovakia	17.2		4.6
Hungary	17.8		13.3
Poland	23.2		0.7
Romania	18.5		8.5
Yugoslavia	9.0	4.4	0.4
USSR	258		
Japan	15.5	47.2	
Turkey	37.0		
Australia	43.7		15.8
New Zealand	12.2		3.3
Peoples Republic of China			30.9

[a] From USDA (1975).

[b] Averages for 1972–1974.

[c] 1 million pounds equals 453.59 metric tons.

[d] Missing value indicates no data available.

basis, such as fall flowers, alfalfa-sweet clover, and "mixed flowers" from various specified areas.

Actually, many recognized types of honey are produced in the United States; a USDA analytical study included 83 different single-source and 93 blends of known composition (identified by the beekeepers) among the 490 samples of honey analyzed (White *et al.*, 1962). Most of them are not available in sufficient

TABLE II

COMMERCIALLY SIGNIFICANT HONEY TYPES AND NATURAL BLENDS OF THE UNITED STATES

Honey	Area of Production
"Clover"[a]	Central, North-Central, East-Central
Sweet clover	Central, North-Central, East-Central
Sweet clover-alfalfa	Intermountain West
Alfalfa	Central and Mountain West, California
Basswood	Mid-Atlantic to Wisconsin
Buckwheat, wild	California
Cotton	Southwest
Fireweed	Oregon
Gallberry	Southeast
Goldenrod	Northeast
Locust, black	Mid-Atlantic, East Central
Mesquite	Southwest
Orange-grapefruit	Florida, California
Sage	California
Sourwood	Virginia, Carolinas
Spanish needle	Central
Star thistle	California, North Central
Tulip tree	Mid-Atlantic to Indiana
Tupelo	Florida
Vetch	California, Oregon

[a] "Clover" may include alsike, white Dutch, crimson, ladino, and red clovers, vetch, and trefoil.

quantity to be of commercial significance. On a local or statewide basis, however, they assume some importance. Table II lists the commercially significant types of domestic honey usually available in the United States. Depending upon seasonal and environmental factors, some variability in color and, to some extent, flavor within any type should be expected; honey is after all a natural product.

Most food manufacturing use of honey requires such large quantities that the choice is restricted to the clover blends or other natural or prepared blends. Table honey, except for the specialty markets where floral type is preserved, is blended for season-to-season uniformity of color and flavor.

2. *Other Countries*

Table I lists average (1972–1974) production in several countries; only those with significant (about 1 million pounds (454,000 kg) foreign trade are listed.

Crane (1975b) lists production estimates and other data for 26 European, 21 Western Hemisphere, 20 Asian, and 19 African countries, and Australia and New Zealand.

With a few exceptions, honey in world trade is specified and priced by color. Experience with typical honey blends available from the exporting countries allows importers to provide suitable types of honey for specific manufacturing or blending uses. Specialty honeys for table use are available from certain areas: heather from the British Isles, northern and western Europe; thyme honey and pine forest honeydew from Greece; acacia from Hungary, Rumania, Yugoslavia; orange from Spain; mild-flavored light alfalfa, white clover, and thistle from Argentina; legumes and rape from Canada. Honeys of more pronounced flavor include the eucalyptus types from Australia. Mexican honeys, sold largely by color as "mixed flowers," are (Willson, 1975): acahual (*Viguiera grammatagrossa*) (extra light amber), and mesquite (light amber); and from Yucatan, tah (Compositae), which is extra light amber to light amber and of pronounced flavor, and dzidzilché (*Gymnopodium antigonoides*), a fragrant light amber honey much used in baking.

B. PRODUCTION METHODS

1. Migratory Beekeeping

Commercial production of honey was made possible by the invention in the mid-nineteenth century of the movable-frame hive and the centrifugal extractor for removing stored honey without destruction of the comb, allowing its reuse. Beekeeping has changed greatly in this century, from being a craft for which every family farm had a few hives for pollination and a sweetener, to our present monocultural agricultural production methods making available vast acreages of such honey sources as orange, cotton, and legumes (for seed production, since modern forage-producing practices largely recommend cutting just before bloom). Many such areas are not suitable for permanent location, further encouraging migratory beekeeping where thousands of colonies are moved thousands of miles, following the blooming crops from south to north.

2. Permanent Locations

Much of the nation's honey comes from wild sources, encouraging much competition for locations where hives can be kept permanently. Such areas must have a succession of pollen and nectar sources as well as sufficiently moderate winters that the cost (in honey or sugar) of keeping the colony alive is not excessive. Many northern beekeepers destroy the colony each fall and begin anew with purchased queens and package bees in the spring. A major beekeeping

industry (concentrated in the South, Texas, and California) is the production and sale of queens and package bees in the early spring. Colonies depleted by removal of bees for sale may be moved north for strengthening and honey production.

3. *Pollination*

Although most of the beekeepers' income arises from sale of honey, a significant fraction, especially in fruit-, nut-, and seed-production areas comes from planned pollination.

Rental prices per colony of bees vary, depending upon such factors as strength of colony, availability, time required, and possibility of a honey crop, from $4–$15 in California (almond, prune, cherry) to $10–$36 in Michigan, Washington, New York, and other fruit-producing areas.

C. HARVESTING

Management of honeybee colonies for maximum honey production is a blend of art and science and is beyond the scope of this review. Details are described by Cale *et al.* (1975). The hive bodies ("supers") containing combs of ripened honey, largely capped over, are removed from the colony, freed of bees, and taken to a central location for extraction. There the cappings of the cells are removed mechanically and the honey is extracted by centrifugation. It may be run directly into 55-gallon drums for shipment to processors or storage or may be cleaned (to a greater or lesser extent) by allowing it to stand to permit extraneous material to rise to the surface (ripe honey has a density of around 1.42) for removal. It may also be strained through coarse (23 mesh) or fine (100 mesh) screens depending upon the needs of the immediate customer. The frequency of removal of supers will depend upon the honey flow and the need to prevent mixture of different floral types.

D. PROCESSING

1. *Why Process?*

Honey immediately after extraction is at its best in terms of flavor and color. It is not suitable for large-scale marketing without further treatment, however, unless the producer has carried out the required processing (which qualifies him as a "producer-packer"). Most producers sell most of their honey to processors who prepare it for marketing and package it.

As extracted, "raw" honey contains extraneous matter such as pollen, bits of wax, variable amounts of sugar-tolerant yeasts, and probably crystals of dextrose

hydrate.* It is thus prone to fermentation unless the moisture content is below 17%; most honey will crystallize in time unless action is taken to prevent it. Processing of honey thus includes controlled heating to destroy yeasts and dissolve dextrose crystals, combined with fine straining or pressure filtration.

2. *Processing Methods*

a. For a Liquid Product. Even though supersaturated with respect to glucose, honey will not granulate for months if correctly processed, handled, and stored. Pressure filtration, introduced by Lothrop and Paine (1934) for honey, improved shelf life of liquid honey by eliminating seed crystals and fine particles of crystallization-inducing substances. Heat exposure, because of the great sensitivity of honey to heat resulting from its acidity, fructose content, and high viscosity, should be limited only to that necessary to accomplish the functions: "melting" (dissolution of dextrose granulation), pumping, filtration, pasteurization, and filling.

Figure 1 diagrams a plant packing 12 million pounds (5.44 million kg) of honey per year. Honey is received from producers in 55-gallon (208 liter) drums (660 lb, 300 kg), classified for color, floral type, flavor, and moisture and held for use. The melter is designed to liquify 24 drums in about 4 hours without exposure to excessive heating. Most of the liquefaction occurs in the tank beneath the oven from which honey is pumped to batch storage. From this point, it is raised to 150°F (65.6°C) by a heat exchanger, passed through a plate-type filter, and cooled to 120°F (49°C) in the heat exchanger before going to a series of holding tanks in the packing area. Total time at 150°F (65.6°C) is about 30 seconds in this operation. The filling lines for liquid pack honey are conventional; care is needed to avoid reseeding the liquid from holdout residues of honey in lines and equipment.

b. For a Solid Product. A semisolid honey product results from the controlled crystallization of some of the dextrose in very fine grain, producing a fondant-like texture. The line for this product is shown on the bottom of the diagram. Not shown is the addition of about 10% of finely crystallized "seed" honey previously prepared by grinding crystallized honey and storing it at 57°F (14°C) for 5–7 days. This is added in the creamer after the batch temperature is reduced to 80°F (27°C). After thorough mixing, the material is filled into retail containers and held at 57°F (14°C) for a week to complete the fine-textured crystallization. The process as described was patented by Dyce (1935); most production is based on it. Details of equipment may be found in the article by Geddes (1964). Townsend (1975) has described several sizes of honey packing lines.

*The terms "glucose" and "dextrose" are equivalent, as are "fructose" and "levulose."

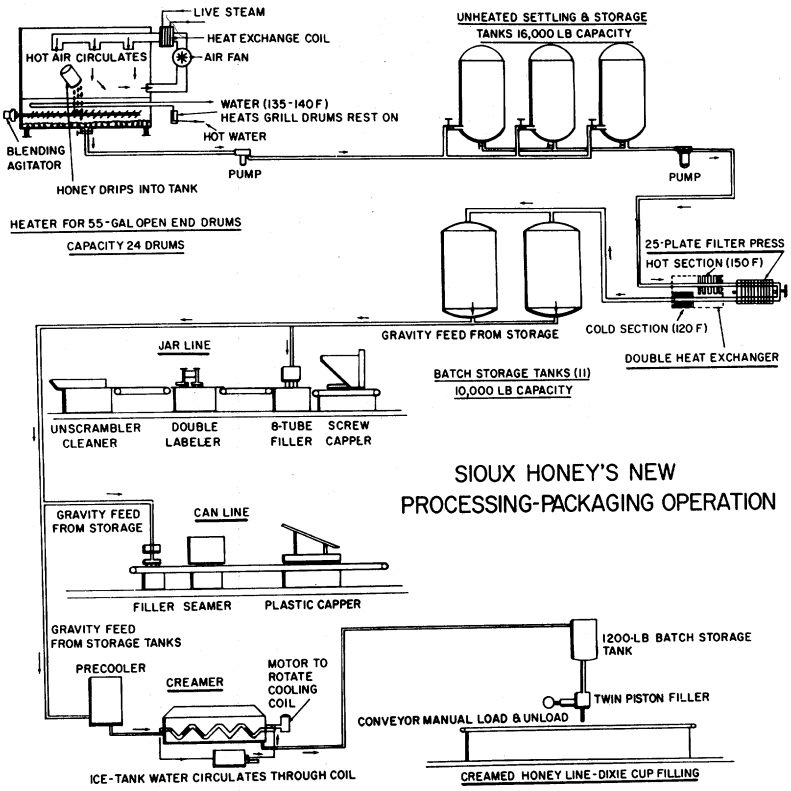


FIG. 1. Layout and flow diagram for commercial honey packing plant with an annual capacity of 12 million pounds. (From Geddes, 1964.)

III. MARKET FORMS OF HONEY

A. RETAIL PRODUCTS

1. Liquid

The United States retail market appears to favor liquid honey, while in many other countries a solid form is preferred. Supermarket exposure seems to require the clear, nonturbid product which results from filtration. A considerable amount of honey is sold in alternate markets such as health food stores, roadside stands, or department or specialty stores. This may not be filtered or processed for clarity and may also be partially granulated, a natural state for honey. Unfortunately, if the honey has been pasteurized, granulation may be coarse and gritty, reducing

the appeal of the product. If the honey has not been heated, however, fermentation may take place unless moisture content is 17% or less.

Containers include clear glass, translucent or opaque plastic, and, in larger sizes, metal. Dispensers such as squeeze bottles or drip-cut servers are sometimes available.

2. *Comb Honey Forms*

Honey in the comb is the ultimate in natural flavor and unprocessed nature. It has virtually disappeared from most urban markets, being difficult and expensive to produce and ship. A sealed comb is a real guarantee of a natural product, exactly as prepared by the honeybee. Market forms include: section comb, a $4\frac{1}{2}$ inch (11.4 cm) square frame which the bees have filled with honey; cut-comb, which is a piece cut by the beekeeper from a larger comb; and chunk-comb or bulk-comb honey, which is a piece of sealed honey comb in a container filled with liquid honey.

3. *Solidified Honey*

Since most honeys are supersaturated in dextrose, the most stable form would seem to be biphasic. The truth appears to be that for nearly all honey there is actually no completely stable form, although for most marketing requirements the liquid form is sufficiently stable. As briefly noted above, honey is also sufficiently shelf-stable for sale in a semisolid form known as "creamed," "spun," "churned," recrystallized, or "honey spread." This is a fondant of fine dextrose hydrate crystals in the honey matrix. It has a "short" consistency and can be spread or handled without the difficulties of a thick syrup. Nothing extraneous is added in manufacture; the product is a result of a controlled crystallization process which follows the normal pasteurization. This is necessary because the liquid portion of the product is somewhat higher in moisture content than before crystallization and hence more liable to fermentation. Storage at temperatures over about 81°F (27°C) will lead to softening and eventually partial liquefaction, since the equilibrium between solid and solution is temperature dependent.

B. PRODUCT FOR MANUFACTURING USE

Honey for use in food, confectionery, and pharmaceutical products is currently available in the liquid form. Generally darker honey types or blends of more pronounced flavor are required to ensure that an identifiable flavor contribution is made to the product. Such types are also somewhat less costly. A mild, light clover type will provide honey attributes other than flavor in a product (hygro-

scopicity, browning of baked goods, "doctoring" or fondants, etc.) but may not contribute its flavor significantly to that of a product with a flavor of its own.

Industrial honey is generally purchased in 55-gallon drums, but tank trucks can be available. It should be purchased on sample and on specification if possible; specification should be concerned only with attributes pertinent to use. Honey must contain, at most, 18.6% moisture, and be of acceptable color and flavor for the intended use, be filtered to assure cleanliness, be processed to remain liquid, and, if needed, to inactivate enzymes (see later). Since the semisolid honey spread retail form is more easily handled at the table without drip and stickiness, it is conceivable that a similar form would be of use in certain food manufacturing operations; it could be handled similarly to solid shortenings. Such a product could be made available by honey processors if demand indicated a need.

IV. ANALYSIS AND COMPOSITION

Honey as produced by honeybees from plant nectars is rather variable in its composition, reflecting contributions of the plant, climate and environmental conditions, and beekeeper skills. Table III summarizes the general composition of United States honey. Data available from similar studies in other countries (White, 1975a) provide similar values.

TABLE III

AVERAGE COMPOSITION OF 490 SAMPLES OF HONEY AND RANGE OF VALUES[a]

Characteristics measured	Average	Standard deviation	Range
Moisture, percentage	17.2	1.46	13.4–22.9
Levulose, percentage	38.19	2.07	27.25–44.26
Dextrose, percentage	31.28	3.03	22.03–40.75
Sucrose, percentage	1.31	0.95	0.25–7.57
Maltose, percentage	7.31	2.09	2.74–15.98
Higher sugars, percentage	1.50	1.03	0.13–8.49
Undetermined, percentage	3.1	1.97	0.0–13.2
pH	3.91	—	3.42–6.10
Free acid, meq/kg	22.03	8.22	6.75–47.19
Lactone, meq/kg	7.11	3.52	0.00–18.76
Total acid, meq/kg	29.12	10.33	8.68–59.49
Lactone/free acid	0.335	0.135	0.000–.950
Ash, percentage	0.169	0.15	0.020–1.028
Nitrogen, percentage	0.041	0.026	0.000–.133
Diastase value	20.8	9.76	2.1–61.2

[a] From White *et al.* (1962).

A. MOISTURE CONTENT

1. Analysis

The amount of water in honey is of major importance to its stability against fermentation and granulation. Normally ripened honey has a moisture content below 18.6%; honey of higher content does not qualify for the USDA grading classifications.

The determination of honey moisture has been reviewed extensively by White (1969); no significant developments have occurred since that time. In that review, moisture determination by direct drying, Karl Fischer reagent, measurement of viscosity, and density by weighing and hydrometry are critically discussed, as are certain errors and inconsistencies in the literature. The most accurate and convenient procedure uses the refractometer with the conversion table recalculated by Wedmore (1955), which appears as method 31.112 of the Association of Official Analytical Chemists (Horwitz, 1975). Approximations suitable for many purposes (standard error ± 0.4%) may be obtained with a hand refractometer, providing proper calibration is used, since sucrose solutions and honey of the same refractive index differ in their solids content. Table IV provides a conversion of solids (sucrose) by refractometer to honey solids.

2. Relation of Moisture Content to Stability

The principal short-term instabilities of honey are granulation and fermentation. Liability to each is related to moisture content: fermentation by osmophilic

TABLE IV

CONVERSION OF REFRACTOMETER CALIBRATION AT 20°C FROM SUCROSE TO HONEY SOLIDS[a]

Sucrose	Honey solids	Refractive index
76.00	77.56	1.4804
77.00	78.56	1.4829
78.00	79.60	1.4855
79.00	80.64	1.4881
80.00	81.68	1.4907
81.00	82.76	1.4934
82.00	83.76	1.4960
83.00	84.80	1.4987
84.00	85.80	1.5014

[a] Calculated from data of Wedmore (1955) and AOAC table 52.012 (Horwitz, 1975).

TABLE V

LIABILITY OF HONEY TO FERMENTATION[a]

Moisture content (%)	Liability
Below 17.1	None
17.1–18.0	None if yeast count <1000/gm
18.1–19.0	None if yeast count <10/gm
19.1–20.0	None if yeast count <1/gm
Above 20.0	Always liable

[a] Data of Lochhead (1933) based on 319 honey samples.

yeasts will ensue if the combination of moisture content, temperature, and yeast count is favorable (Lochhead, 1933); granulation tendency appears to be fairly predictable by the glucose/water ratio (White *et al.*, 1962; Hadorn and Zürcher, 1974). Normally ripened honey with a moisture content of 17.5–18%, with a water activity of 0.58, requires a natural inoculum of about 1000/gm to ferment (Lochhead, 1933). Table V shows the general relation between moisture content and yeast count in honey liable to fermentation. USDA (optional) standards (USDA, 1951) require that honey contain no more than 18.6% water to qualify for grading. Retail honey is usually blended to 18% water or less.

A survey of composition of United States honey (White *et al.*, 1962) showed that honey from the Western and intermountain areas is lower in moisture than that from the East and West North Central areas, with other United States areas intermediate (average 17.5% for 238 samples). Exact values will be affected by seasonal factors.

B. CARBOHYDRATES

The sugars of honey have been intensively reviewed recently by Siddiqui (1970), White (1975b), and Doner (1977). For that reason, the discussion here will be limited, including only a description of our present understanding of the carbohydrate composition of honey and its analyses.

1. Average Composition and Ranges

Table III lists the amounts of glucose, fructose, sucrose, "maltose" (reducing disaccharides), and higher sugars found in a survey of nearly 500 samples of United States honey. The variability of honey is illustrated by the ranges shown. A better conception of this is seen in Fig. 2 which illustrates the distribution of individual values within the range for these sugars. Individual analyses for these sugars (and other components) are given by White *et al.* (1962) for 504 honey

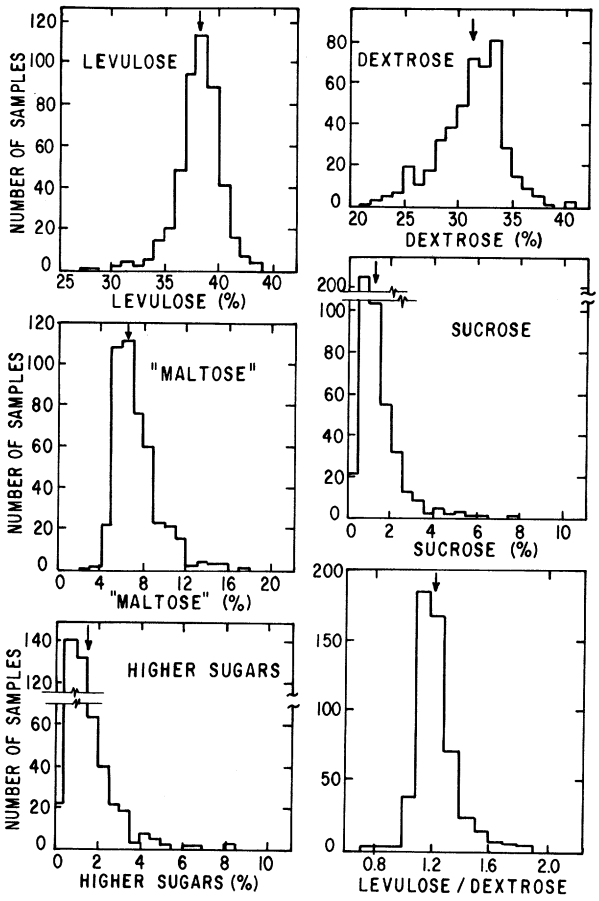


FIG. 2. Distribution of carbohydrate contents among 490 honey samples. Arrows indicate means. (From White *et al.*, 1962.)

and honeydew samples from 47 states, representing 83 single floral types, 93 blends of "known" composition, and 4 honeydew types, all from two crop years.

The routine paper chromatography carried out as a control in the fractionation procedure indicated that all samples had the same pattern of sugars present. Also in the publication, the carbohydrate (and other) composition of 74 honey types was compared with the average values. Effects of area of production were examined for alfalfa, cotton, and orange honeys produced in widely different areas. Any differences found were not significant.

It is noteworthy that only 3 of the honey samples had a levulose:dextrose ratio

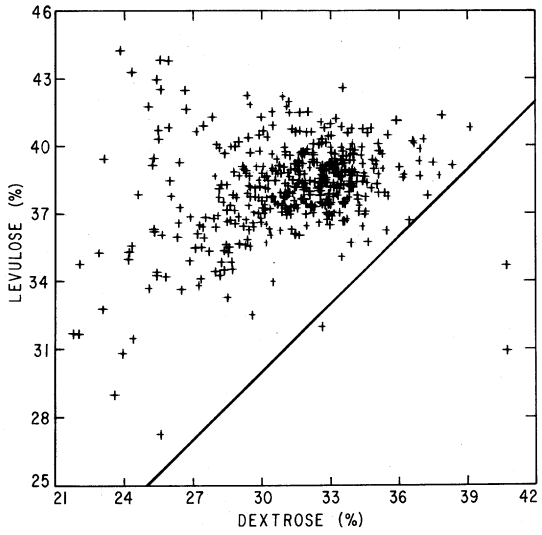


FIG. 3. Dispersion of monosaccharide content of 457 honey samples; line indicates L/D = 1. (Data of White *et al.*, 1962.)

less than 1.0. Figure 3 shows the individual values of this ratio found for 457 honey samples. The floral source has the strongest influence on carbohydrate composition; area and seasonal influences are minor.

2. *Identity of Sugars*

Sugars which have been unequivocally identified in honey are listed in Table VI. Identification requires isolation and identification by sound physical or chemical methods of analyses, not simply by comparison of chromatographic mobility.

Details of isolation and identification are included in the reviews of Siddiqui (1970) and Doner (1977). Table VII provides an approximation of the amounts of the oligosaccharides found in honey by Siddiqui and Furgala (1967, 1968a).

3. *Analytical Problems*

In common with other syrups, the carbohydrate analysis of honey remained empirical for many decades. Not until White and Maher (1954) applied class separation on charcoal columns was a reasonably accurate method available for determining dextrose and levulose in honey; three fractions are obtained. This method, accepted by the AOAC, remains the method of choice; it is somewhat

TABLE VI

SUGARS ESTABLISHED AS HONEY CONSTITUENTS[a]

Trivial name	Systematic name	Reference
Glucose		
Fructose		
Sucrose	α-D-glucopyranosyl-β-D-fructofuranoside	
Maltose	*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose	Elser (1924); van Voorst (1941)
Isomaltose	*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose	White and Hoban (1959)
Maltulose	*O*-α-D-glucopyranosyl-(1→4)-D-fructose	White and Hoban (1959)
Nigerose	*O*-α-D-glucopyranosyl-(1→3)-D-glucopyranose	White and Hoban (1959)
Turanose	*O*-α-D-glucopyranosyl-(1→3)-D-fructose	White and Hoban (1959)
Kojibiose	*O*-α-D-glucopyranosyl-(1→2)-D-glucopyranose	Watanabe and Aso (1959)
Laminaribiose	*O*-β-D-glucopyranosyl-(1→3)-D-glucopyranose	Siddiqui and Furgala (1967)
α,β-Trehalose	α-D-glucopyranosyl-β-D-glucopyranoside	Siddiqui and Furgala (1967)
Gentiobiose	*O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose	Siddiqui and Furgala (1967)
Melizitose	*O*-α-D-glucopyranosyl-(1→3)-*O*-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside	Siddiqui and Furgala (1968a)
3-α-Isomaltosylglucose	*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→3)-D-glucopyranose	
Maltotriose	*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose	Siddiqui and Furgala (1968a)
1-Kestose	*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranosyl-(1→2)-β-D-fructofuranoside	Siddiqui and Furgala (1968a)
Panose	*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose	Siddiqui and Furgala (1968a)
Isomaltotriose	*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose	Siddiqui and Furgala (1968a)
Erlose	*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-β-D-fructofuranoside	Siddiqui and Furgala (1968a)
Theanderose	*O*-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-β-D-fructofuranoside	Siddiqui and Furgala (1968a)
Centose	*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-D-glucopyranose	Siddiqui and Furgala (1968b)
Isopanose	*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose	Siddiqui and Furgala (1968a)
Isomaltotetraose	*O*-α-D-glucopyranosyl-(1→6)-[*O*-α-D-glucopyranosyl-(1→6)]$_2$-D-glucopyranose	Siddiqui and Furgala (1968a)
Isomaltopentaose	*O*-α-D-glucopyranosyl-(1→6)-[*O*-α-D-glucopyranosyl-(1→6]$_3$-D-glucopyranose	Siddiqui and Furgala (1968a)

[a] From Doner (1977).

TABLE VII

YIELDS OF THE PRINCIPAL SUGARS IN THE OLIGOSACCHARIDE FRACTION (3.65%) OF HONEY[a]

Disaccharide	(%)	Trisaccharide	(%)	Higher oligosaccharide	(%)
Maltose	29.4	Erlose	4.5	Isomaltotetraose	0.33
Kojibiose	8.2	Theanderose	2.7	Isomaltopentaose	0.16
Turanose	4.7	Panose	2.5		0.49
Isomaltose	4.4	Maltotriose	1.9		
Sucrose	3.9	1-Kestose	0.9	*Acidic Fraction*	
Maltulose, (and		Isomaltotriose	0.6	Not investigated	6.51
2 unidentified		Melizitose	0.3		
ketoses)	3.1	Isopanose	0.24		
Nigerose	1.7	Centose	0.05		
α,β-Trehalose	1.1	3-α-Isomaltosyl-			
Gentiobiose	0.4	glucose	trace		
Laminaribiose	0.09				
	56.99		13.69		

[a] Data of Siddiqui and Furgala (1967, 1968a).

lengthy if many analyses are required. Recent development of high-performance liquid chromatographic (HPLC) analyses of sugar mixtures (Conrad and Palmer, 1976) promises a more rapid procedure without loss of accuracy. The several gas chromatographic (GLC) procedures are of intermediate value, suffering as they do from the need to derivatize.

A large degree of empiricism remains in the charcoal column procedure, however. Sucrose can be analyzed specifically only if yeast invertase hydrolysis is used; melezitose is eluted in the disaccharide fraction and interferes if acid hydrolysis is used. The remaining mixture of reducing disaccharides is measured by reducing power and reported as "maltose." It is too complex to allow individual sugars to be quantitated, even by HPLC. All oligosaccharides (other than melezitose) are present in the higher sugars fraction, reported as glucose after hydrolysis.

Literature reports of the sugar composition of honey must be examined with a knowledge of the analytical procedures employed. Values obtained by polarimetric or saccharimetric means are only roughly approximate; those using specific methods for either glucose or fructose and calculation of the other by difference are not accurate unless a class separation has first been done. Values from GLC analysis for the monosaccharides are usually acceptable; for sucrose and other sugars they may not be since demonstration of the singular nature of peaks, considering all of the honey sugars, has not been done.

Data from HPLC may be quite acceptable for monosaccharides and sucrose, but all other disaccharide peaks should be combined as "maltose," since the

chromatograms do not reflect the known complexity of the honey sugars. Improvements in columns and detectors may eventually provide analyses of more components.

C. ACIDS

The characteristic flavor of honey (if such a variable commodity can be said to have a characteristic flavor) includes a contribution due to its acidity. The pH of honey (Table III) averages 3.91, with a range for 490 samples of 3.42 to 6.10. This level of active acidity probably also contributes to the stability of honey against microbiological attack.

1. Gluconic as the Principal Acid

Gluconic acid, in equilibrium with gluconolactone, is the principal acid of honey (Stinson *et al.*, 1960). It is produced by the action of the glucose oxidase normal to honey on the glucose (White *et al.*, 1963b). This reaction is extremely slow in full-density honey but rapid when honey is diluted. It has been proposed that this acid is produced from nectar glucose during the ripening of nectar to honey by the bee. The combined effect of acidity and the hydrogen peroxide concurrently produced is thought to assist in preserving nectar from spoilage during the ripening. Burgett (1974) has shown that this also occurs in nine other eusocial Hymenoptera.

2. Other Acids

Ten other organic acids have been identified in honey by suitably rigorous procedures, and seven more are probably present. The former group includes acetic, butyric, lactic, and pyroglutamic (Stinson *et al.*, 1960), citric and succinic (Nelson and Mottern, 1931), formic (Vogel, 1882, cited by Farnsteiner, 1908), maleic (Goldschmidt and Burkert, 1955), malic (Hilger, 1904), and oxalic (von Philipsborn, 1952). In the latter group are glycollic, α-ketoglutaric, and pyruvic (Maeda *et al.*, 1962), tartaric (Heiduschka and Kaufmann, 1913), and 2- or 3-phosphoglyceric acid, α- or β-glycerophosphate, and glucose-6-phosphate (Subers *et al.*, 1966).

3. Analysis

The titration of total acidity of honey had been an empirical procedure because of a fading endpoint. This was shown to be caused by hydrolysis of gluconolactone (White *et al.*, 1958); the present procedure measures free acid and lactone. Of 490 samples of United States honey, only two were found not to contain

gluconolactone; their pH values were uncommonly high: 5.01 and 6.10 (White *et al.*, 1962).

The amount of gluconic acid in honey should be a reflection of several contributing factors, the most significant being the time between the collection of the nectar by the bee and the attainment of full density in the comb, since the action of glucose oxidase essentially stops at full density. This is governed by the sugar content of the nectar, the weather, the strength of the colony, and the quality (*i.e.*, density and volume) of the nectar flow. A greater time needed for ripening permits production of more gluconic acid; it also results in more manipulation of the ripening honey by the bees, with addition of more enzyme. A need exists for an analytical procedure to determine total gluconic acid and gluconolactone in honey. Present lactone titration is not satisfactory because the position of the lactone-acid equilibrium, as related to honey pH, is not known.

D. MINERAL CONTENT

1. Average Amounts of Principal Minerals

The wide variability of honey composition is reflected also in the ash content. Table III shows an average of 0.17%, with a range from 0.02–1.03%. The predominating mineral element is potassium, which averages about one-third of the ash; sodium content is roughly one-tenth as much. Schuette and his students at Wisconsin (Schuette and Remy, 1932; Schuette and Huenink, 1937; Schuette and Triller, 1938; Schuette and Woessner, 1939) published the data summarized in Table VIII. They found honey in the two lightest color classes to have lower mineral content than the darker honey types. This was confirmed by White *et al.* (1962).

2. Trace Minerals

The literature on the content of these minerals and 13 others in honey from other areas of the world was reviewed by White (1975a). Tong *et al.* (1975), in an examination of the value of trace analysis of honey as an indicator of pollution, reported ranges for 41 elements in 19 New York State honey samples. Samples collected by bees in the vicinity of the New York Thruway appeared to contain elevated levels of elements known to be emitted by internal combustion engines.

E. PROTEINS AND AMINO ACIDS

The nitrogen content of honey is low and variable. Table III shows an average for United States honey of 0.041%, with a standard deviation of 0.026 (63%).

TABLE VIII

MINERAL CONTENT OF HONEY[a]

Mineral element	Honey color	No. samples	As parts per million of honey: Range	Average
Potassium (K)	light	13	100–588	205
	dark	18	115–4,733	1676
Sodium (Na)	light	13	6–35	18
	dark	18	9–400	76
Calcium (Ca)	light	14	23–68	49
	dark	21	5–266	51
Magnesium (Mg)	light	14	11–56	19
	dark	21	7–126	35
Iron (Fe)	light	10	1.20–4.80	2.40
	dark	6	0.70–33.50	9.40
Copper (Cu)	light	10	0.14–0.70	0.29
	dark	6	0.35–1.04	0.56
Manganese (Mn)	light	10	0.17–0.44	0.30
	dark	10	0.46–9.53	4.09
Chlorine (Cl)	light	10	23–75	52
	dark	13	48–201	113
Phosphorus (P)	light	14	23–50	35
	dark	21	27–58	47
Sulfur (S)	light	10	36–108	58
	dark	13	56–126	100
Silica (SiO_2)	light	10	7–12	9
	dark	10	5–28	14

[a] Data of Schuette and Remy (1932), Schuette and Huenink (1937), Schuette and Triller (1938), and Schuette and Woessner (1939).

Paine *et al.* (1934) reported an average of 55% of the nitrogen lost by ultrafiltration (range 26–93%); White and Kushnir (1967b) noted that about 40–60% of the nitrogen is lost on dialysis. Bergner and Diemair (1975) more recently reported 33–45% to be removed by ultrafiltration (10,000 limit). Most of the nonprotein nitrogen is in free amino acids.

1. Proteins

Early interest in protein content was in distinguishing honey from artificial mixtures and blends. The volume of precipitates with honey and tannin (Lund, 1909), phosphotungstic acid (Lund, 1910), or alcohol (Laxa, 1923) was used. Immunological tests were studied as early as 1903 (Langer, 1915). Thöni (1913) proposed using an antiserum to royal jelly or "beebread" for this purpose.

Indeed, Langer (1915) immunologically differentiated honey protein and proteins of hand-collected pollen, refuting Küstenmacher's earlier claim that protein in honey was extracted from the pollen and that Langer was in error in ascribing it to the bee.

Studying the colloidal material removed from honey by ultrafiltration, Paine *et al.* (1934) found it to be more than half protein, isoelectric at pH 4.3, and precipitable by colloidal bentonite. Helvey (1953) found three components in the colloidal material from a buckwheat honey: proteins of molecular weight of 146,000 and 73,000 and a presumed polysaccharide of 5,000 weight. White and Kushnir (1967b), using gel filtration, ion-exchange chromatography, and starch-gel electrophoresis, examined proteins of eleven floral types of honey and sugar-fed stores. From four to seven proteins were found, of which four originate with the bee. The molecular weights of two of the latter were approximately 40,000 and 240,000; those from the plant were about 98,000 and >400,000. Figure 4 shows gel filtration and starch-gel electrophoresis of a preparation from

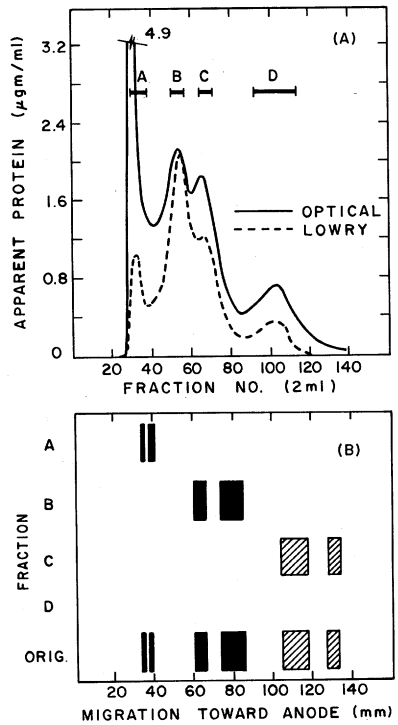


FIG. 4. (A) Filtration of 1 ml dialyzed concentrate (≅ 10 gm honey) goldenrod-aster honey on 2.1 × 60-cm column of Sephadex G-200 in 0.01 *M* phosphate pH 6.5; (B) Starch gel electrophoresis of fractions combined in pH 8.9 borate, 4.0 V/cm as indicated in A. Pattern at bottom is from original material as applied to column. (From White and Kushnir, 1967b.)

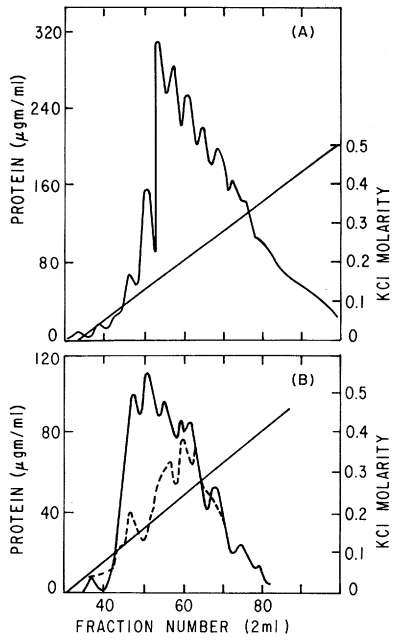


FIG. 5. DEAE-cellulose chromatography (bed 0.8 × 18 cm) in 0.01 *M* phosphate (pH 8.0) of concentrated dialyzed protein preparations. Solid lines: apparent protein by optical method. Broken lines: by Lowry method (scales left). Straight solid lines: gradient of concentration (scale right). (A) Goldenrod-aster preparation, 0.25 ml, (B) *Lespedeza,* from single comb, 1.0 ml. (From White and Kushnir, 1967b.)

a goldenrod-aster honey. In Fig. 5 is seen the greater resolution produced by ion exchange cellulose chromatography of this preparation. The chromatogram of a preparation from sugar-fed bees (no nectar components) is shown in the same figure to indicate its less complex nature. In Fig. 6 are gel filtrations of protein preparations from another honey and from stores from sugar-feeding. The larger number of components in the former is apparent.

Bergner and Diemair (1975) have also examined by gel filtration protein preparations from several types of honey and from sugar-feeding. Their results have generally confirmed those of White and Kushnir. They ascribed three of the five elution peaks to the bee and two to plant components.

2. *Amino Acids*

The formol titration, essentially a measure of total amino acid content, was applied to honey by Tillmans and Kiesgen (1927) who proposed that it be used to

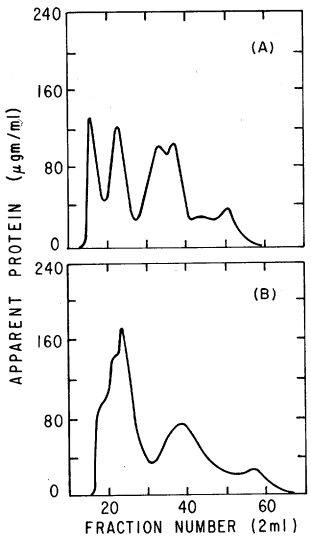


FIG. 6. Filtration of concentrated dialyzed honey preparations (0.5 ml) on Sephadex G-200. Protein by Lowry method. (A) cotton honey, (B) stores from sugar-fed bees. (From White and Kushnir, 1967b.)

authenticate honey. European limits for this value were shown by Schuette and Templin (1930) to be inapplicable to United States honeys, which were generally lower and more variable. Lothrop and Gertler (1933) described a procedure for amino nitrogen in honey, reporting an average of 0.0033% (range 0.0024–0.0066%). Schuette and Baldwin (1944) reported averages of 0.0034% for light and 0.0058% amino nitrogen for dark honeys. The introduction of paper chromatography renewed interest in honey amino acids; several investigators identified up to 17 amino acids in various samples. Komamine (1960), quantitating paper chromatography, first noted that proline was the preponderant amino acid. Later the automatic amino acid analyzer was used for honey analyses; a considerable body of analyses is now available (Curti and Riganti, 1966; Mizusawa and Matsumuro, 1968; Michelotti and Margheri, 1969; Hahn, 1970; Biino, 1971; Bergner and Hahn, 1972; Petrov, 1974; Davies, 1975). Table IX shows Davies' values for free amino acids in 98 honey samples.

All agree that proline predominates, representing 50–85% of the total. Davies (1975) has reviewed the sources of honey amino acids. Since pollen contains about 1.5% amino acids, with proline predominating, Komamine proposed this as the source. Nectar contains small amounts of free amino acids but little proline. Davies calculated that far too little pollen is present in honey to account for the proline. Bergner and Hahn (1972), noting proline to comprise 80% or more of the generally lower amino acid content of sugar-fed bee stores, ascribed

TABLE IX

AMINO ACID ANALYSIS OF HONEYS[a,b]

No. samples:	Argentina 8		Australian eucalypt/-clover 16		Australia unspecified 15		Canadian 16		U.S. clover 13		Yucatan 14		All samples 98	
	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
Glucosaminic acid							3.17	1.664					3.21	1.688
Methionine sulphoxide	1.14	1.241	2.38	1.268	1.44	0.955			0.59				1.74	1.174
Aspartic acid	1.76	0.587	1.24	0.577	1.47	2.169	2.05	0.245	4.50	1.463	9.90	1.592	3.44	3.212
Unknown A	0.64	0.218			0.46								0.95	0.937
"Amides"	6.48	2.961	7.57	1.120	5.09	3.439	6.68	0.561	14.82	4.532	30.90	4.673	11.64	9.334
Glutamic acid	2.12	0.809	1.66	0.245	1.51	1.981	2.02	0.296	5.73	1.831	6.00	0.643	2.94	2.163
Proline	53.10	16.589	93.95	16.658	50.49	25.424	51.81	6.954	44.96	16.170	83.42	23.930	59.65	26.765
Unknown B											13.54	3.691	21.04	20.612
Glycine	0.55	0.176	0.54	0.169	0.43	0.190	0.43	0.102	0.71	0.115	1.48	0.208	0.68	0.407
Alanine	1.73	0.679	2.42	0.673	1.21	0.632	1.16	0.121	1.85	0.482	3.84	0.601	2.07	1.523
Cystine			0.66						0.30	0.125	0.45	0.179	0.47	0.212
Valine	1.27	0.346	1.43	0.318	0.92	0.372	1.32	0.248	1.32	0.481	6.16	1.545	2.00	1.854
Methionine	0.07		0.25	0.090	0.33	0.223	0.97				0.09		0.33	0.232

Isoleucine	0.72	0.279	0.73	0.170	0.47	0.229	0.75	0.317	0.69	0.278	3.82	0.961	1.12	1.191
Leucine	0.69	0.267	0.64	0.203	0.88	0.753	0.59	0.288	0.54	0.205	2.89	0.514	1.03	0.898
Tyrosine	0.91	0.391	1.29	0.382	2.24	1.689	1.04	0.420	1.13	1.280	7.26	2.176	2.58	2.826
Phenylalanine	2.07	1.525	3.17	1.241	5.07	4.253	1.76	0.204	3.35	3.757	60.49	13.800	14.75	24.806
β-Alanine	0.98	0.452	1.57	0.400	1.29	0.260	0.74	0.223	0.66	0.387	1.00	0.402	1.06	0.510
γ-Amino butyric acid	1.34	0.318	2.76	0.770	1.62	0.810	1.52	0.207	1.30	0.421	4.23	0.617	2.15	1.186
Unknown C	0.63	0.681	1.49	0.242	1.19	0.538	0.41	0.156	0.37	0.113	0.87	0.275	0.81	0.513
Unknown D	0.30	0.068			0.77	0.176			0.25	0.148	0.61	0.092	0.66	0.476
Unknown E	0.33	0.175	0.69	0.082	0.64	0.225	0.26	0.115	0.37	0.327	0.63	0.244	0.57	0.442
Lysine	1.32	0.768	0.44	0.206	0.34	0.118	1.28	0.337	1.06	0.393	1.97	0.486	0.99	0.666
Unknown F	0.64		0.34	0.106	0.41	0.191			0.31	0.139	0.93	0.440	0.50	0.359
Ornithine	0.24	0.064	0.20	0.084	0.27	0.191	0.12	0.131	0.25	0.112	0.43	0.172	0.26	0.223
Histidine	6.04	2.305	2.70	0.728	1.85	0.398	5.42	0.602	4.83	1.238	5.64	0.838	3.84	1.982
Tryptophan	2.06						1.91	1.431	3.83	0.986	6.17	0.996	3.84	3.393
Arginine	0.82	0.437	1.30	0.690	1.12	0.600	0.86	1.238	1.12	0.767	6.05	3.372	1.72	2.269
Total:	84.10	26.484	127.96	19.857	77.25	33.976	83.88	7.530	90.46	26.601	252.28	41.846	118.77	69.491

[a] From Davies (1975).

[b] mg amino acid/100 gm honey (dry wt).

[c] A blank in the SD column indicates that only one sample contained the amino acid.

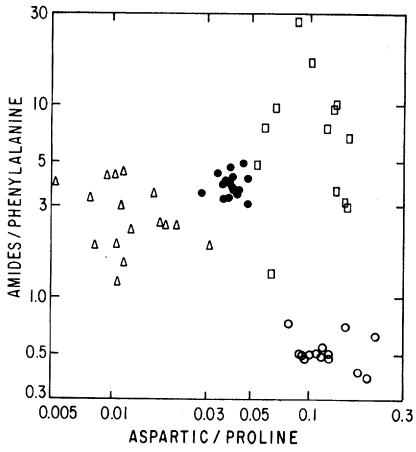


FIG. 7. Regional separation of honeys by ratios between concentration of individual amino acids. △ Australian, ● Canadian, □ United States clover, ○ Yucatan. (From Davies, 1975.)

it to the bee. Petrov related it to the important part proline plays in aerobic muscle exchange products in all insects.

Davies, using data for 98 samples of honey, has suggested that certain ratios between contents of various amino acids could be used to determine the geographic source of a honey; Fig. 7 indicates one such approach. Later (Davies, 1976) this approach was refined by using a computer-aided selection of 60 amino acid ratios. Fifteen of 16 samples not used to establish the program were correctly assigned to one of the four locations shown in Fig. 7, showing that while there are variations in the ratios between samples of the same area, the variation between sources is much greater.

F. ENZYMES

That honey contains enzymes has been known for more than a century since Erlenmeyer and Planta (1874) reported their presence in bees, pollen, beebread, and honey. As the author has noted earlier,

> The enzymes are among the most interesting materials in honey, possibly have received the greatest amount of research attention over the years, and have supported the greatest burden of nonsense in the lay and even scientific press. The use of enzyme activity in some countries as a test for overheating of honey seems to support by implication the occasional supposition by food faddists that the enzymes of honey have a dietetic or nutritional significance of themselves (White, 1975a).

The greatest volume of literature reports on honey enzymes until most recently dealt with their use as indicators of honey identity and quality, largely heat

TABLE X
ENZYME ACTIVITIES OF HONEY

Enzyme	Average activity	Number of samples	References
α-Glucosidase (invertase, sucrase)	7.5–10	1468	Duisberg and Hadorn (1966)
Diastase (α- and β-amylase)	16–24	1483	Duisberg and Hadorn (1966)
	20.8	263	White *et al.* (1962)
Glucose Oxidase	80.8	90	White and Subers (1963)
	167	24	Dustmann (1971a)
	210	10	Dustmann (1971b)
Catalase	4.97[a]	28	Schepartz and Subers (1966a)
	86.8[b]	10	Dustmann (1971b)
Phosphatase	13.4	25	Dzialoszyński and Kuik (1963)
	5.07		Zalewski (1965)

Units

α-Glucosidase:	gm sucrose hydrolyzed per 100 gm honey per hour at 40°C
Diastase:	gm starch converted per 100 gm honey per hour at 40°C
Glucose oxidase:	μg H_2O_2 accumulated per gm honey in 1 hour under experimental conditions. Because honey contains substances oxidized by H_2O_2, this is not a true measure of glucose oxidase
Catalase:	Catalatic activity per gram, $K_f = 1/t\ (\ln x_0/x)\ D/W$ where x_0 is initial substrate, x is substrate at time, t, D is dilution, and W is sample in grams (Schepartz and Subers, 1966a)
Phosphatase:	mg P/100 gm honey/24 hours

[a] Includes nine values of zero.
[b] Includes four values of zero.

exposure. Most countries other than the United States require minimum values for amylase activity and proposals for use of other enzyme activities for this purpose still arise.

The honey enzymes of most direct interest in food applications are amylase, invertase, and glucose oxidase. Catalase and acid phosphatase are also present. The amounts of these enzymes normally found in (unheated) honey are shown in Table X, to provide an idea of order of magnitude.

1. Invertase

A sucrose-splitting enzyme is added to nectar by the honeybee during its harvesting and ripening to honey. It continues its activity in extracted honey unless destroyed by heating. It is an α-glucosidase (White, 1952; White and Maher, 1953a) with inherent transglucosylase action. During its action on su-

crose, six oligosaccharides are formed, all eventually hydrolyzed to glucose and fructose by the completion of the reaction. The principal intermediate is α-maltosyl β-D fructofuranoside (White and Maher, 1953b) trivially named erlose (also termed glucosucrose, fructomaltose). It can accumulate to as much as 11% of the original sucrose (White and Maher, 1953b) during the reaction. Maltose is formed in lesser amounts. Echigo and Takenaka (1973) have studied the carbohydrates and α-glucosidase in stores produced by sucrose-fed caged bees; during the ripening they reported erlose to appear in the earlier part of the ripening period and remain throughout the ripening period. Figure 8 shows the progress of ripening of sucrose stores. The optimal conditions for the transferase reaction were found to be pH 6.0, 30°C, and 0.25 *M* sucrose.

Examination of α-glucosidase from several honeys and from stores of sucrose-fed bees (White and Kushnir, 1967a) indicates that preparations seemingly homogeneous by Sephadex gel filtration (Fig. 9) show 3–9 components by ion-exchange chromatography. The preparation from sugar-fed bees, however, appeared homogeneous with an approximate molecular weight of 51,000 indicated. Figure 10 illustrates DEAE cellulose chromatography of α-glucosidase from honey and of stores from sugar-fed caged bees. A high-resolution starch gel electrophoresis procedure (White and Kushnir, 1966) further resolved all preparations into 7–18 isozymes. Figure 11 compares the pattern of α-glucosidase isozymes from a bulk honey (i.e., extracted from combs taken from many colonies at several locations) with that of the α-glucosidase from a comb honey (i.e., produced by a single colony) and that of stores from sugar-fed bees.

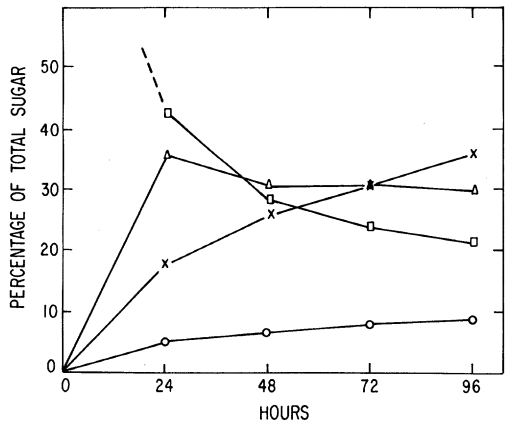


FIG. 8. Changes in sugar content during the process of honey formation. × fructose, △ glucose, □ sucrose, ○ erlose. (From Echigo and Takenaka, 1973.)

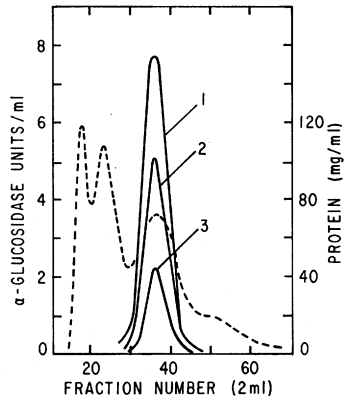


FIG. 9. Sephadex (G-200) filtration (2.1 × 31 cm) in 0.014 *M* phosphate (pH 6.5) of enzyme preparations from honey (solid lines, scale left): α-glucosidase from (1) 0.5 ml preparation (≅ 1.1 g) goldenrod-aster honey; (2) 0.5 ml preparation (≅ 5.5 g) clover honey; (3) 0.25 ml preparation from (≅ 8.9 gm) stores from sugar-fed bees. Broken line (scale right) from same clover honey. (From White and Kushnir, 1967a.)

The greater complexity of the preparation from bulk honey is probably the result of blends of honey from many colonies. The single-colony samples have equivalent numbers of isozymes. Noteworthy is the much lower migration rate of the sugar-fed samples, which have no plant components. White and Kushnir suggest that the bands may represent genetic differences among bees. Methods

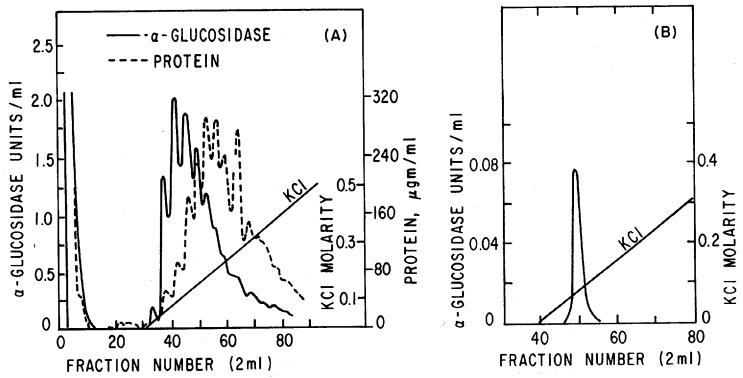


FIG. 10. (A) Chromatography on DEAE-cellulose of: α-glucosidase preparation from cotton honey elution with 0.01 *M* potassium phosphate (pH 8.0); KCl gradient as shown; solid line: α-glucosidase activity (scale left); broken line: "protein" measured by optical method (scale right). "Protein" retained and fractionated, 14.9 mg (73%); α-glucosidase retained and fractionated, 73.5 units (27%); (B) α-glucosidase preparation from stores of sugar-fed bees. (From White and Kushnir, 1967a.)

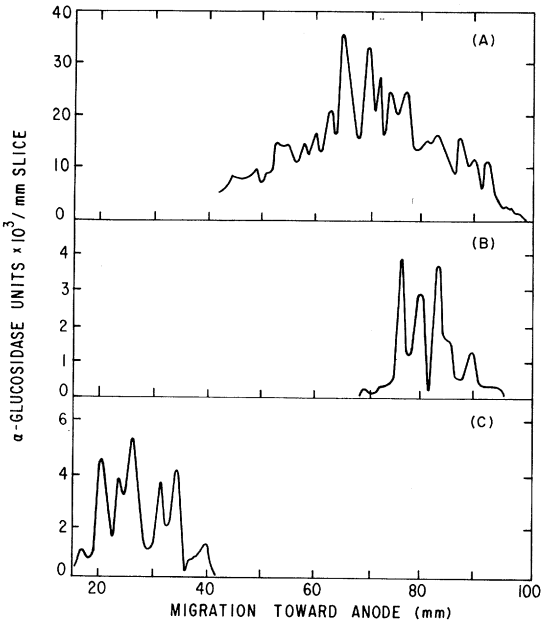


FIG. 11. Starch-gel electrophoresis of α-glucosidase preparations from honey, borate (pH 8.9): (A) clover honey, 96 units/ml; (B) *Lespedeza* honey, 38 units/ml; (C) stores from sugar-fed bees, 44 units/ml. A at 3.70 V/cm, B and C at 3.52 V/cm. (From White and Kushnir, 1967a.)

used are sufficiently sensitive to examine the α-glucosidase of single bees in this fashion.

a. Origin and Kinetics of Honey Invertase. The question of the source of the sucrose-inverting enzyme of honey has intrigued scientists since its discovery in honey. No purpose is served by reviewing the earlier literature; it has been accepted for many years that the major portion is that added by the bee during the collection of nectar and the ripening process. Whether any plant enzyme from nectar or pollen is present has not been definitively shown. Gothe (1914) concluded that both plant and insect were sources, since more enzyme activity was present in honey than in stores from sugar-feeding. Schönfeld (1927), however, found the invertase activity of sugar-fed stores to be inversely related to the concentration of the feed; no information is available on the concentration fed by Gothe.

The kinetic study of honey invertase by Nelson and his colleagues (Nelson and Cohn, 1924; Nelson and Sottery, 1924; Papadakis, 1929) remained the definitive work until recently. Differences from yeast invertase were found in pH optima and the initial reaction course, initial yeast invertase rates being practically

constant, in contrast to a marked rate increase in the honey invertase inversion.

Two kinetic studies with the objective of determining the source of honey invertase provide the only recent kinetic data on the reaction.

Rinaudo *et al.* (1973) undertook to demonstrate that invertases from the other possible sources (pollen, nectar) differ from that of honey. Invertase from the hypopharyngeal gland of the bee and from honey were shown to have the same pH and temperature sensitivity, substrate and reaction products (glucose and fructose; intermediates were not mentioned), and inhibition by fructose. Comparison of reciprocal rate plots for the enzymes from the bee, honey, two pollens, and nectar showed identical Michaleis constants (0.17 *M*) only for the first two. However, they reported that none of the preparations showed maltase, contrary to earlier reports (White and Maher, 1953a; Gontarski, 1954; Maurizio, 1961).

The definitive study of the α-glucosidase of the hypopharyngeal gland of the honeybee (and hence of honey) is that of Huber. Huber (1975) and Huber and Mathison (1976) have purified two sucrases from honeybees, confirming Gontarski's (1954) earlier studies. The less soluble of these precipitated between the same values of ammonium sulfate saturation and exhibited kinetics very similar to those of honey sucrase. Final purification was by affinity chromatography. The previously reported transglycosylase activity was confirmed and a kinetic study of the hydrolytic and synthetic reactions was carried out. As seen in Fig. 12, the rate of release of fructose is rectilinear; the rate of glucose release drops at

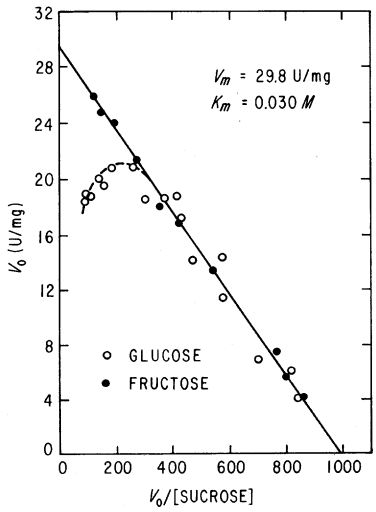


FIG. 12. Kinetic Hofstee plot of the production of glucose and fructose from sucrose. For incubation details see original. Units are micromole product per minute from 0.2 *M* sucrose at 30°, pH 6.5. From Huber and Mathison, 1976. Reproduced by permission of the National Research Council of Canada from the *Canadian Journal of Biochemistry* **54,** 153–164, 1976.

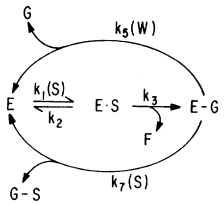


FIG. 13. Proposed mechanism for action of the major sucrase of honey bees; S, sucrose, G, glucose, F, fructose, G-S trisaccharide, W, water. From Huber and Mathison, 1976. Reproduced by permission of the National Research Council of Canada from the *Canadian Journal of Biochemistry,* **54,** 153–164, 1976.

high sucrose concentrations. The scheme in Fig. 13 explains this: the reaction releasing fructose (K_3) is rate limiting, and sucrose is not only initial substrate, but an acceptor for the transglucosylation to form the glucose-sucrose trisaccharide, erlose. Huber did not identify the trisaccharide with erlose. The K_{cat} for fructose, glucose, and erlose formation according to the scheme are:

$$K_{\text{cat (fructose)}} = K_3 \tag{1}$$

$$K_{\text{cat (glucose)}} = \frac{K_3 K_5(W)}{K_5(W) + K_7(S)} \tag{2}$$

$$K_{\text{cat (erlose)}} = \frac{K_3 K_7(S)}{K_5(W) + K_7(S)} \tag{3}$$

K_M for formation of all three products by this mechanism is K^2/K^1. (K_{cat} values which include substrate concentrations are not, in fact, constants, since the values change with substrate concentrations.)

The earlier value of 51,000 for the molecular weight (obtained by Sephadex filtration in 0.1 *M* phosphate pH 6.5) was confirmed by equilibrium ultracentrifugation, but Huber and Mathison found values of 82,500 by Sephadex filtration (0.2 *M* citrate pH 6.5) and 78,000 by SDS electrophoresis. The glycoprotein nature of the enzyme was confirmed by amino acid and amino sugar analysis. Honey α-glucosidase has been reported to have optimal maximum activity at pH 6.0 (10% hydrolysis), 5.7 (35–55% hydrolysis) (Nelson and Cohn, 1924), 5.9 (Rinaudo *et al.,* 1973), and 5.5 (Huber and Mathison, 1976). Hadorn and Zürcher (1962), comparing several published procedures for determination of sucrase in honey with particular attention to the pH optimum, selected 6.3, commenting that very little difference in activity was found between pH 5.8–6.5. Huber and Mathison, who used the most highly purified enzyme, show a flat maximum between pH 5.5 and 6.0.

This discrepancy in K_M values reported for the major honeybee enzymes (and for honey sucrase) by Rinaudo *et al.* (1973) (0.17 *M*) and Huber and Mathison (1976) (0.030 *M*) may perhaps be resolved by a calculation of K_M for honey

invertase using the data published by Nelson and Cohn (1924). A Lineweaver-Burk plot of the 5-minute data from their Table 10 gives $K_M = 0.031\ M$. Considering the crude nature of their preparation, this is excellent agreement.

When comparing data among the few recent reports on these α-glucosidases, the sources and extent of purification must be considered. Huber and Mathison prepared their material from whole honeybees, necessitating extensive purification. They also prepared the enzyme from an unpasteurized supermarket honey. The latter was purified by dialysis and ammonium sulfate precipitation. They stated that the kinetic properties and apparent K_M values of the honey enzyme were the same as the "major honeybee sucrase." The sucrase of the head portion of the honeybee is almost entirely of this type. Rinaudo *et al.* (1973) prepared honey sucrase by dialysis, and that from excised honeybee food (hypopharyngeal) glands and pollen by extraction and centrifugation only. No estimates of purity were made. The hypopharyngeal glands are known to contain an active glucose oxidase (Gauhe, 1941) which, if not removed, can distort results by preferentially removing glucose. This glucose oxidase activity was probably eliminated in Huber's preparation from bees, and it was probably present in those of Rinaudo. It is not clear, however, whether the less extensive purification given the honey enzyme by Huber effectively removed the glucose oxidase known to occur in honey. Huber makes no mention of glucose oxidase.

There is no question that the α-glucosidase of honey acts upon maltose; no reason is apparent for the contrary report of Rinaudo *et al.* (1973). Huber and Mathison (1976) report activity against maltose to be 83% that against sucrose. White and Kushnir (1967a) show exactly parallel activity for 13 isozyme peaks separated by starch-gel electrophoresis; the activity against maltose was only 30% of that against sucrose. Takenaka and Echigo (1975) purified honey α-glucosidase by DEAE cellulose chromatography and obtained K_M for maltose $= 0.00526\ M$, with optima at pH 6.0 and 30°C. Sucrose was hydrolyzed at three times the rate for maltose.

b. Heat Inactivation. The papers of Rinaudo *et al.* (1973) and of Huber and Mathison (1976) include data on heat inactivation of the α-glucosidase in buffer. The latter report only that the activity rapidly disappears at 55°–65°C; Huber and Mathison state that with 10-minute exposure destruction begins at 40°C and is essentially complete at 60°C.

Trade interest in the invertase activity of honey centers about its possible use as an indicator of heating history. Most of the literature on the subject deals, therefore, with inactivation in full-density honey. White *et al.* (1964) have shown that the rate constant for inactivation of honey invertase in buffer is 24 times that in full-density honey.

Several proposals have been made to establish minimum values for sucrase in honey, to be used together with diastase values for estimating heat exposure

(Kiermeier and Köberlein, 1954; Duisberg and Gebelein, 1958; Hadorn *et al.*, 1962). The Codex Alimentarius standards, however, do not include sucrase activity. No discussion of analytical methods will be included here, beyond the observation that Dustmann (1972) pointed out that existing methods in which the reaction takes place in diluted honey (including those of the preceding three papers, above) underestimate the activity by 10–30%, presumably because of the inhibitory effect of the monosaccharides of honey. He recommended dialysis as a pretreatment. The procedure earlier used by White *et al.* (1964) in their study of the effect of storage and processing on honey enzymes had used dialysis for this reason. In that study, a single expression was found adequate to describe the inactivation of invertase in full-density honey by heating and long-term storage. The first-order rate equation is

$$\log K = 26.750 - \frac{39730}{2.303\ RT} \tag{4}$$

which is plotted in Fig. 14. Figure 15 shows the half-life of honey invertase over the temperature range of 20°–80°C.

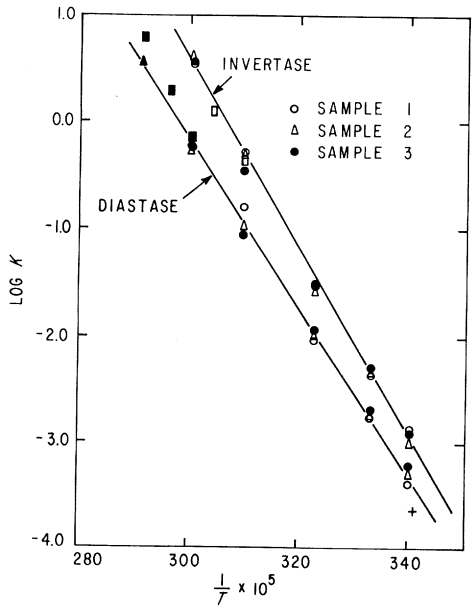


FIG. 14. Effect of temperature on rate of heat inactivation of diastase and invertase in honey; + Schade *et al.* (1958), ■ Lampitt *et al.* (1929), □ Duisberg and Warnecke (1959), ▲ Kiermeier and Köberlein (1954). From White *et al.*, 1964. Reprinted from Food Technology/*Journal of Food Science* **18**(4), 153–156, 1964. Copyright © by Institute of Food Technologists.

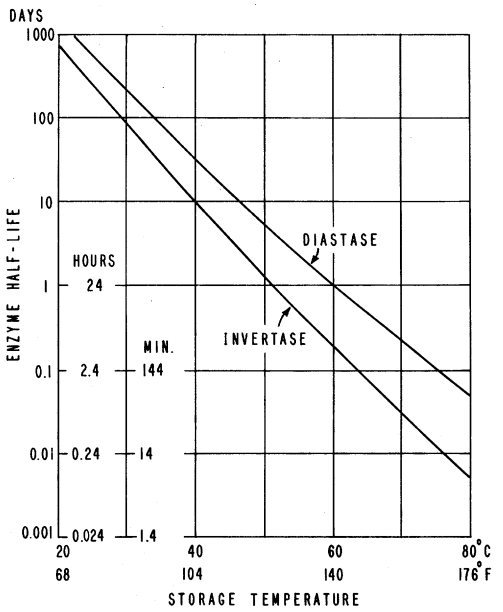


FIG. 15. Approximate time required at a given temperature between 20°C (68°F) and 80°C (176°F) for the diastase and invertase activities of a honey sample to be reduced to one-half of the initial value. (From White, 1967.)

2. *Amylase*

For an enzyme whose occurrence in honey has been known for 100 years, with hundreds of reports written on methods of quantitation, factors affecting activity in honey, and reporting assays of many thousands of honeys, very little is known of its kinetics, mode of action, and indeed, the significance of its presence in honey.

a. Isolation and Purification. The importance attached to amylase assay as a quality factor in honey is indicated by its inclusion in the Codex Alimentarius standards for honey. This reflects the preference of consumers in many countries for honey with relatively minor exposure to heat. Establishment of minimum acceptable values for honey "diastase" provides a control procedure. Since a few honey types are known that are naturally deficient in diastase, special provision is made for exceptions. Kerkvliet and van der Putten (1973) have compared five methods for determining the diastatic activity of honey by measuring the loss of iodine-coloration power.

TABLE XI

SUMMARY OF ANALYTICAL DATA ON THE PREPARATION OF AMYLASE FROM HONEY[a]

Preparation stage	Total diastase units	Total α-amylase units	Protein (mg)	Diastase / mg protein	α-Amylase / mg protein	Diastase / α-amylase
Original honey (200 gm)	6520	220.0	3180.0	2.0	0.07	29.6
Dialysed solution	6797	229.0	1663.0	4.1	0.14	29.7
Acetone precipitate	4590	148.0	731.0	6.3	0.20	31.0
Amm. sulph. precipitate	1853	59.0	134.0	13.8	0.44	31.4
DEAE (cellulose) bands:						
A	292	31.0	2.2	130.0	13.80	9.4
B	247	8.2	66.2	3.7	0.12	30.1

[a] From Schepartz and Subers (1966b).

Honey has both α- and β-amylase activity; both the increase in reducing sugar and loss of coloration with iodine have been used in assays. The latter is most commonly used. Lampitt *et al.* (1929) reported optimal pH for the α-amylase to be about 5.0 in the 22°–30°C temperature range, and 5.3 between 45°–50°C. For β-amylase, a value of 5.3 was reported.

Schepartz and Subers (1966b) attempted to separate the α- and β-amylase activities of honey by several procedures. Used as the final isolation procedure, ion-exchange cellulose chromatography provided two principal fractions. Table XI summarizes the research. Efforts to characterize the pooled fractions were unproductive because of their instability; the presence of α-glucosidase further complicated interpretation of the results. A 200-fold purification of the α-amylase was attained.

White and Kushnir (1967a) carried out Sephadex gel filtration of dialyzed honey concentrates and determined amylase activity on the fractions. As seen in Fig. 16 a single peak was obtained using maleate buffer, indicating an approximate molecular weight of 21,600. Interaction with the Sephadex is evident when phosphate buffer is used with three maxima in the elution curve. Bergner and Diemair (1975), however, obtained single elution peaks in phosphate (pH 5.3) from G-200, as seen in Fig. 17, but considerably more retarded than the glucosidase peak in maleate, similar to the highest peak in phosphate in Fig. 15.

b. Heat Inactivation. Diastase has been used for at least 75 years as an indicator of honey heating. Nearly all of the reports are therefore oriented to this aspect, as is true for the invertase. Most of this work has measured α-amylase activity. The few papers reporting β-amylase inactivation in honey must be discounted because the α-glucosidase and glucose oxidase in honey may vitiate the results by their effect on maltose or glucose from the amylolytic reaction.

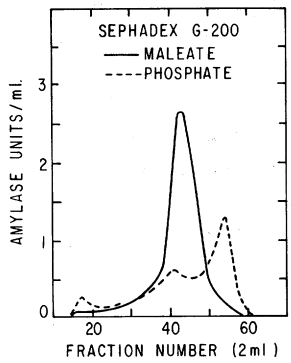


FIG. 16. Sephadex (2.1 × 31 cm) filtration of enzyme preparation from cotton honey in 0.01 *M* maleate (pH 6.5), and in 0.01 *M* phosphate (pH 6.5). (From White and Kushnir, 1967a.)

The most complete study of heat inactivation of diastase (α-amylase) in honey remains that of White *et al.* (1964), who showed that loss of diastase by heating and by extended storage at lower temperatures obey first-order kinetics and can be described by the equation

$$\log K = 22.764 - \frac{35010}{2.303\ RT} \tag{5}$$

as shown in Fig. 14, which also includes data from other investigators. Figure 15 provides an estimate of the half-life of diastase in honey within the temperature range 20°–80°C.

It should be pointed out that although the relationship between time, temperature, and invertase and diastase activity have been widely quoted by honey

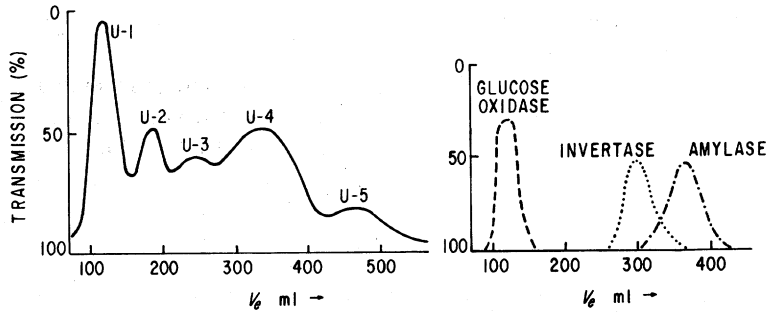


FIG. 17. Elution diagram of 10 ml protein concentrate on Sephadex G-200 (2.3 × 83.5 cm) in 0.03 *M* phosphate (pH 5.3) after freezing (−20°C) and remelting. (From Bergner and Diemair, 1975.)

scientists and control officials, they are based on a study of only three United States honeys (White *et al.*, 1964). This work should be extended to include other representative honeys.

c. Source of Honey Amylase. In contrast to the α-glucosidase, which has a clear and essential function in the conversion of nectar to honey, no such function has been assigned to the starch-digesting enzymes in honey. Nectar contains no starch or dextrins. The question of its origin, in view of this, has been examined for many years.

The presence of definite amounts of diastase in stores from sugar-fed bees (diastase numbers about 10 or less) led Gothe (1914) and others to ascribe it mostly to the bee, with a contribution from pollen. Vansell and Freeborn (1929) later contended that pollen, known to have diastatic activity, was the principal source, and Lothrop and Paine (1931) supported this, citing the great variation in diastase value among honeys of different floral type. On the other hand, Fiehe (1932) considered nectar to be the major source; most honey has diastase numbers considerably in excess of the lower values common to stores from sugar-feeding. Braunsdorf (1932) found diastase numbers of 17.9 in two sugar-fed samples and proposed that it originates largely from the bees, with the variability resulting from the different degree of manipulation by strong or weak colonies upon slow or heavy flows of nectar. Weishaar (1933) ascribed only 1.5–2.5% of the diastase to nectar, 0.25–0.75% to pollen, and the remainder to the bee. Rinaudo *et al.* (1973) considered honey amylase originating from the bee as the basis of an optimal pH of 5.6–5.9 for preparations of the enzyme from honey and from honeybee hypopharyngeal glands. Nectar amylase pH optimum was 7.2, but that from pollen at pH 5.9 did not differ appreciably from that of honey. Amylase from honey and the bee was activated by chloride ion, in contrast to that of pollen and nectar.

If diastase originates largely in the food glands of the honeybee, as does α-glucosidase, it would be expected that the ratios of these two enzymes in honey be relatively constant. Since α-glucosidase is more heat (and storage) labile, values for unheated honey should be used.

The writer has calculated the correlations between diastase number and sucrase number of 39 unheated samples from the literature: 30 Swiss honeys from Table 1 of Hadorn *et al.* (1962), 4 United States honeys from Table 2 of the same paper (actually supplied to the authors by the writer), and 5 stores from sugar-feeding, described in Table 1 from Hadorn and Zürcher (1963). A correlation coefficient of $+0.83$ resulted with $F = 26.2$, significant at less than 0.01%.

There appears to be little doubt that the major portion of the diastase in honey originates from the bee, and the variability probably reflects the specific conditions during gathering and ripening of the nectar.

3. *Glucose Oxidase*

Honey has been thought from ancient times to have wound-healing and antiseptic properties, and within the past 40 years a distinct heat-labile antibiotic activity has been the subject of considerable interest. The activity was named "inhibine" and a biological test was devised for its measurement in honey (Dold *et al.,* 1937). During analytical studies on honey, the writer and his colleagues found that the drifting end-point common in the determination of acidity in honey, ascribed by Cocker (1951) to an acid-producing enzyme, was actually caused by the hydrolysis of lactone material in honey (White *et al.,* 1958). Further studies indicated gluconic acid, in equilibrium with gluconolactone, to be the principal honey acid (Stinson *et al.,* 1960).

With the knowledge that a glucose oxidase had been reported in the hypopharyngeal glands of honeybees (Gauhe, 1941), it was demonstrated that the enzyme was present in honey and its production of gluconic acid and hydrogen peroxide during the standard microbiological test for inhibine was responsible for the major part of the antibiotic effect (White *et al.,* 1963b).

A chemical assay was described (White and Subers, 1963) in which the accumulation of hydrogen peroxide in diluted honey during a 1-hour incubation was measured colorimetrically. From the results on 45 samples assayed by the Dold *et al.* (1937) plate assay and by the chemical assay, a relation between the inhibine number and log of peroxide accumulation was found. The effect of heating honey for 10 minutes at 70°C on the inhibine number and on peroxide accumulation was investigated for 29 samples, and for 6 the half-life of the peroxide accumulation system was determined (White and Subers, 1964a). A wide range in stability was found: most samples lost 85–95% of the activity when heated 10 minutes at 70°C, but seven lost less (6–71%) and five lost more (96–100%). Figure 18 shows a comparison of the heat sensitivity of the peroxide accumulation system in six honeys with that of honey diastase and invertase. The great variability, which precludes its use an index of heating exposure, is obvious.

In a study of the previously reported instability of inhibine to light, White and Subers (1946b) found a wide variation in this effect; some honeys lost 90% of the activity on exposure to normal laboratory fluorescent light for 1 hour, others lost only 10% in full sunlight for 10 minutes. The sensitivity is maximal at 425–525 nm and pH 3, and is negligible at pH 6–7; a heat- and light-stable, nonvolatile sensitizer was postulated.

The use of inhibine number or glucose oxidase activity as a measure of honey quality on heat exposure is therefore impractical because of the wide range of activity and the wide range (70-fold) of heat sensitivity shown by authentic honeys.

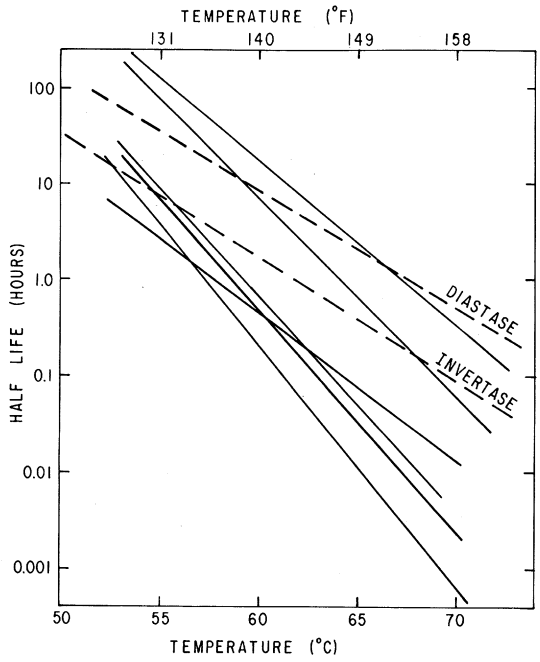


FIG. 18. Effect of temperature on the half-life of the peroxide accumulation system in honey. Diastase and invertase shown for comparison. (From White and Subers, 1964a.)

Schepartz and Subers (1964) and Schepartz (1965a,b, 1966a) have examined the glucose oxidase of honey. The enzyme is a true glucose oxidase, aerobically transferring H_2 directly to molecular oxygen. Its pH optimum is 6.1 and requires 0.1 *M* Na^+ for maximal activity. Of 32 carbohydrates tested, only glucose (100%) and D-mannose (9%) were oxidized. The optimal temperature is 40°C; it is completely inactivated at 60°C. It is strongly inhibited by NaCN and semicarbazide, somewhat by EDTA, mannose, fructose, and azide. Heavy metals at 0.001 *M* did not inhibit. The enzyme shows a preference for β-D-glucose over α-D-glucose of about 6:1. A kinetic study (Schepartz, 1965b) revealed the unusually high optimum substrate concentration of about 2.7 *M* (equilibrium glucose) as indicated in Fig. 19A. The Michaelis constant is 1.49 *M*, shown in Fig. 19B. In terms of β-D-glucose, the optimal substrate concentration is 1.8 *M*. The reaction follows zero order kinetics and is stoichiometric.

Further investigation of the apparent contradictory action of fructose as an inhibitor or activator under certain conditions was carried out by Schepartz (1966a). Using manometric procedures, the results in Fig. 19 were obtained using varying concentrations of glucose (0.5–2.7 *M*) and fructose (0.1–2.2 *M*).

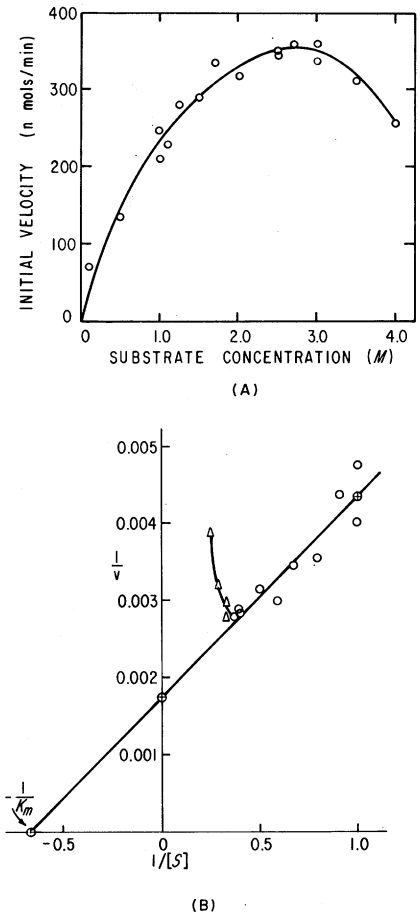
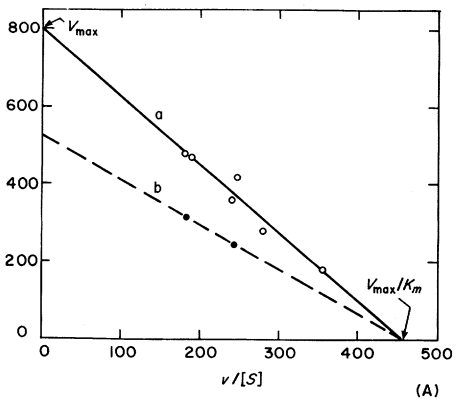


FIG. 19. (A) Effect of substrate concentration in velocity of reaction of honey glucose oxidase with glucose, measured manometrically. For details see original. (B) Lineweaver-Burk plot. Points were derived from those in A; v, initial velocity, (S), substrate concentration, K_m, Michaelis constant, ○, points included in statistical analysis, △ points not included in statistical analysis since beyond optimum concentration, ⊕, points derived from statistics and used to locate line. (From Schepartz, 1965b.)

The plotted data in Fig. 20A suggest a coupling or uncompetitive inhibition by fructose. The plot of the transposed data from the experiments in which both sugars were present by the method of Hunter and Downs (1945) yielded the hyperbolic curve in Fig. 20B, which is found, according to Webb (1963), in the rare instance of coupling or uncompetitive inhibition wherever the inhibitor combines only with the enzyme-substrate complex, never with the enzyme alone.



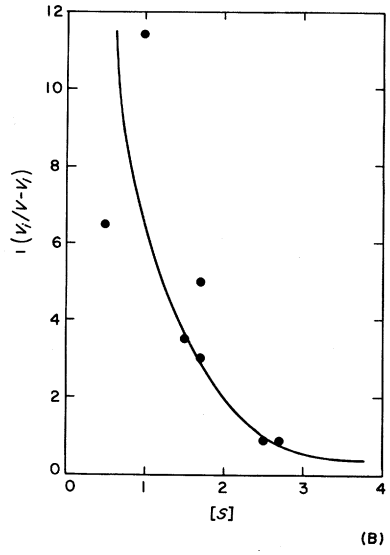


FIG. 20. (A) Plot of velocity-substrate data. In (a) the complete system contained: 0.5 to 2.7 ml 3.5 *M* glucose in 0.2 *M* sodium phosphate (pH 6.1), and enough of the same buffer to total 3.4 ml in the main space; 0.1 ml (419 units) enzyme preparation in a side-arm sac; 0.2 ml 10% KOH in the center well. In (b) the system was the same except 1.7 *M* fructose was present in the main space at the 1.7 *M* and 1.0 *M* glucose levels. Blanks were run without enzyme, without substrate; *v*, initial velocity in μ mole/min, *(S)*, substrate concentration in *M*, v_{max}, maximum velocity, K_m, Michaelis constant. (B) Plot of transposed velocity-substrate data. Conditions same as in A, except that fructose concentration was varied from 0.1 to 2.2 *M;* the combined sugar concentrations never exceeded 3.4 *M*. (From Schepartz, 1966a.)

Webb states that this gives rise to circumstances in which the inhibitor can cause activation.

There is little doubt that honey glucose oxidase originates in the bee. Gauhe (1941) has shown that the glucose oxidase of the hypopharyngeal gland of the honeybee also has a high optimal substrate (about 2 *M*) and a high Michaelis constant (K_M 0.63), greatly in contrast to those of other reported glucose oxidases such as the K_M of 0.0042 for a mold enzyme reported by Keilin and Hartree (1948). Bee and honey glucose oxidases have pH optima at 6.1 and 6–7, respectively, and are equally specific for glucose. Most of the few other glucose oxidases oxidize a number of substrates.

The great variability among honeys of peroxide accumulation, related to the antibiotic effect, does not imply a corresponding variability in glucose oxidase content of honey. The peroxide accumulation assay is carried out with diluted honey so that any constituents oxidized by peroxide will depress the value found. Thus data such as those of White and Subers (1963) showing a thousandfold variability in peroxide accumulation cannot be cited as indicating that the honeybee is not the source of the enzyme. To the writer's knowledge, no true assays of glucose oxidase in honeys have been reported.

4. Other Enzymes

Catalase and an acid phosphatase are the remaining enzymes demonstrated to occur in honey. Gontarski (1948) described a "vitamin C oxidizing enzyme" in the hypopharyngeal glands of bees and observed a similar action in honey. He proposed that it might be identical with the glucose oxidase Gauhe (1941) reported in the bee glands. It is now apparent that this enzyme is in fact glucose oxidase; Schepartz (1966a) showed ascorbic acid to be a powerful activator of honey glucose oxidase by way of product removal and not due to action on the enzyme itself.

a. Catalase. Schepartz (1966b) has reviewed critically the eight reports of catalase in honey which have appeared since Auzinger first reported it in 1910. Because of the earlier use of inappropriate methods and inconclusive experiments, he rejected them and, using manometric and spectrophotometric procedures, has claimed the first unequivocal evidence for catalase in honey. Using a dialyzed honey solution, he found a pH optimum at 7–8.5, a Michaelis constant of 0.0154 *M*, and an optimum substrate concentration at 0.018 *M* H_2O_2, with the reaction being first order. Subsequently Schepartz and Subers (1966a) described a kinetic assay procedure and reported catalase values for 28 honeys. Since diastase and peroxide accumulation values were available for the same samples, correlations were calculated. A direct correlation ($r = -0.76$, sig. at 0.01 probability level) was found between diastase and catalase, and as expected, an inverse

correlation ($r = -0.71$, sig. 0.01) between catalatic activity and peroxide accumulation. In the latter calculation, however, 12 samples which showed little or no catalase but also had little or no peroxide accumulation were not included. Inclusion of these samples reduces the correlation to 0.11, significant at less than the 90% probability level. It is evident that catalase activity is but one of the factors contributing to variability in peroxide accumulation. Dustmann (1971b) provides further evidence; he assayed 11 samples for catalase, using Schepartz's procedure, and also for peroxide accumulation. The four samples with extremely high peroxide values (380–662) were totally devoid of catalatic activity. Dustmann's other catalase values (46.1–241) are all greatly higher than those of Schepartz (0.5–17.8); the same procedure and units appear to have been used. Calculations by the writer based on the seven samples with catalatic activity showed a correlation coefficient between catalase and peroxide accumulation of 0.023 (not significant). When all samples were included, the correlation coefficient is −0.71 (sig. at .05). Using the assay procedures that Schepartz has since declared inapplicable (Schepartz, 1966b), Gillette (1931) reported source of catalase to be pollen. Dustmann (1971b), in the only study using acceptable procedures, has found very high catalase activity for pollen, very little in nectar. No reports could be found of catalase assay of stores of sugar-fed bees on which acceptable assay methods were used.

b. Phosphatase. Giri (1938), on the basis of the production of inorganic phosphorus from β-glycerophosphate during a 24-hour incubation at 35°C with diluted honey, stated that honey contains an acid phosphatase. The activity was maximal at pH 4.5–6.5 and was increased by magnesium ions. His two (of eleven) most active samples were "slightly fermented"; Giri stated that fermented honey samples were characteristically high, and values for unfermented samples were decidedly low, and it is lowered somewhat by pasteurization. He suggested that it is "derived chiefly from fermentation yeast and bees and partly from the plants." Günther and Burckhart (1967) described an improved procedure requiring a 3-hour incubation with *p*-nitrophenylphosphate.

Zalewski (1965) assayed honey, pollen, nectar, and bees for acid and alkaline phosphatases using disodium phenyl orthophosphate as substrate, with incubation of 2.25 hours at 37°C for honey and nectar, 18–24 hours for pollen. Chloroform was added to control microbiological action. Acid phosphatase activity in honey and nectar ranged between 30–2140 and 15–2750 μmole/100 gm dry weight, respectively. Stores from caged sugar-fed bees had about $^1/_{16}$ of the honey average. The acid phosphatase assay of pollen ranged from 1260–145,500 μmole/100 gm. It is implied that pollen is the principal source, although it is apparent that nectar contains sufficient to account for the activity in honey. Whether the enzyme can pass through the wall of the intact pollen grain is debatable.

G. FLAVOR AND COLOR

1. Flavor

Table honey is attractive to the consumer for a variety of reasons, flavor possibly being the most significant. While there seems to be a characteristic "honey flavor," the wide variety of flowers attractive to bees overlays a great multiplicity of source-specific flavors and aromas. Color is also variable and strongly influenced by source, but more susceptible to environmental factors than is flavor. Flavors are sufficiently distinctive that dozens of different floral types can be identified by flavor alone by the experienced taster.

Typical flavors can range from the most delicate and desirable to some that are harsh and objectionable. Generally, though not invariably, the lighter colors are associated with the milder, more pleasant flavors.

The flavor complex includes, in addition to the volatile aromatic materials dominating sweetness, contributions from the acids, traces of polyphenolics, amino acids, and in some cases specific bitter or characteristic nonvolatile notes.

2. Aroma

Relatively little attention has been given to the volatile aroma constituents. Gas-liquid chromatography has been applied by several groups of investigators (Dörrscheidt and Friedrich, 1962; ten Hoopen, 1963; Cremer and Riedmann, 1964). As is true of most natural products examined in this way, the lower aliphatic aldehydes, ketones, alcohols, and esters make up the bulk of the identified components. Cremer and Riedmann identified over half of 120 compounds separated by a 1 mm × 100 m Golay column and observed, after long storage, increases in pentanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and *n*-propanol, suggesting that these may arise from the corresponding amino acids. Sixteen of the 22 honeys examined contained phenylethyl alcohol, and 14 also had benzyl alcohol. It is noteworthy that most synthetic honey flavors contain large amounts of lower aliphatic esters of phenylacetic acid and phenylethylsalicylate or phenylacetate; Jacobs (1955) states that nearly all phenylacetic esters are characterized by a honeylike taste and odor.

An example of a type-specific aromatic is methyl anthranilate (MA), reported in citrus honey by Lothrop (1932). Lavender honey also contains it (Hadorn, 1964). Knapp (1967) has elaborated upon White's (1966) suggestion that MA content may be a useful quality measure for citrus honey, commenting that only 1 of 1000 samples would be expected to contain less than 1.5 ppm. He proposed that additional work be done with known single-source citrus samples as needed. The 80 predominately citrus samples reported by the two investigators averaged 3.8 ppm MA, range 0.84–4.9. Twelve noncitrus samples analyzed by White

averaged 0.07 ppm (range 0–0.28). Data for Knapp's 14 noncitrus samples were not available.

3. *Color*

Little is known of the specific compounds responsible for the color of honey. Of 92 honeys Browne (1908) analyzed, 25 gave a positive test for polyphenolic compounds with $FeCl_3$; the most intense reactions were from the darkest honey. Milum (1939) ascribed the increase in color of honey upon storage to reaction of iron from processing equipment and containers with polyphenols, the browning reaction of reducing sugars and amino acids, and the instability of fructose in acid solution. Von Fellenberg and Rusiecki (1938) found water-soluble coloring materials to increase with honey color more than did fat-soluble colors.

H. VITAMINS

Honey has measurable amounts of six vitamins but at such low levels that they have no nutritional significance. Table XII summarizes the significant data.

Widely conflicting reports of the ascorbic acid content of honey have been ascribed to interfering materials in the chemical determination. Most honeys contain less than 5 mg/100 gm. Some reports of values as high as 390 mg/100 gm by chemical means should be discounted, but Griebel (1938) confirmed chemical values for mint honey of 160–280 mg/100 gm with bioassays in which 1 gm honey/day protected guinea pigs, corresponding to 100–200 mg/100 gm. High ascorbic acid content (118–240 mg/100 gm) of Iranian honey was reported by Rahmanian *et al.* (1970) by chemical analysis and TLC of derivatives, and also confirmed by bioassay, which indicated levels of 75–150 mg/100 gm. They

TABLE XII

VITAMIN CONTENT IN MICROGRAMS PER 100 GRAMS OF HONEY

Samples	Riboflavin	Pantothenic acid	Niacin	Thiamine	Pyridoxine	Ascorbic acid
29 Minnesota[a]	61	105	360[b]	5.5	299	2400
38 U.S. and Foreign[a]	63	96	320[b]	6.0	320	2200
21 U.S. 3–7 years old[c]	22	20	124	3.5	7.6	—
19 U.S. 1–2 years old[c]	26	54	108	4.4	10.0	—
4 India[d]	12–54	—	442–978	8–22	—	2000–3400

[a] Haydak *et al.* (1942).

[b] Corrected from original data in publication as later shown (Haydak *et al.*, 1943).

[c] Kitzes *et al.* (1943).

[d] Kalimi and Sohonie (1965).

proposed use of this specific honey type (of unknown floral source) for helping relieve marginal vitamin C deficiency often found in Iran.

I. TOXIC CONSTITUENTS

Since tremendous numbers of organic compounds are synthesized by various plants, many with substantial physiological activity, it is inevitable that some may, on occasion, be found in honey. The remarkable aspect is that, as widely as bees forage, the instances of toxic reactions are so few. White (1973) has reviewed the subject in some detail. Perhaps the best-known toxins are those of honeys from the *Ericaceae* (*Rhododendron, Azalea, Andromeda, Kalmia* spp.), with literature descriptions reaching back to Xenephon's description of the mass poisoning of the expedition of Cyrus in 401 B.C. in Asia Minor, presumably by honey from Rhododendron; instances still occur in that area. Other areas from which reports of intoxication from *Ericaceae* honeys are USSR, eastern and Pacific Northwest United States, and Japan. Beekeepers are largely aware of the problem and take appropriate steps to avoid it. Other toxic honey types are those from the tree tutu of New Zealand (actually a honeydew), henbane (*Datura metel*), *Datura stramonium* and *Hyoscyamus niger,* yellow jasmine (Jessamine), euphorbia, and arbutus. Details of toxicology, compounds responsible, and other aspects are in the review by White (1973).

V. PHYSICAL CHARACTERISTICS

The physical attributes of honey are largely conferred by the high concentrations of sugars that compose most of the solids. Viscosity, refractive index, and specific gravity are so closely related to solids content that each has been used to measure moisture (solids) content. Refractive index is the most easily used, as implied in Table IV. A complete table of refractive index-moisture content equivalents appears in the Book of Methods, AOAC (Horwitz, 1975).

Specific gravity (20/20°) varies regularly with moisture content, between 1.4404 at 14.0% moisture through 1.4174 at 18.0% to 1.3550 at 21.0%. A table at 0.2% intervals is available (Wedmore, 1955). Because of the fairly wide natural range, care must be taken to mix thoroughly when blending honeys of different moisture content to avoid layering.

A. RHEOLOGY

Much early effort was expended in attempts to determine moisture of honey with such instruments as the hydrometer (Chataway, 1933) and the falling-ball viscosimeter (Chataway, 1932; Oppen and Schuette, 1939) with approximately

the accuracy, but without the facility, of the refractometric measurement. Using absolute viscosity values, Lothrop (1939) found a rather wide variation among honeys adjusted to equivalent moisture contents. Munro's (1943) data (Table XIII) are the most extensive available.

The high viscosity of honey is most apparent when draining containers or in pumping or processing it. Although Munro stated that most of the decrease of viscosity on warming takes place from room temperature to about 30°C, his observation was based on a linear plot. Pryce-Jones' (1953) plot of Munro's data as log viscosity versus $1/T$ shows that rate of change is relatively constant; only the extent of heating needed to obtain the required viscosity reduction should be applied to minimize heat-induced damage to color and flavor. MacDonald (1963) has examined the effect of temperature on the flow of honey through pipes under a constant head. Table XIV shows the results. For the average of all four pipe

TABLE XIII

VISCOSITY OF HONEY

Type	Moisture content (%)	Temperature (°C)	Viscosity (poise)
Sweet clover[a]	16.1	13.7	600.0
(*Melilotus*)		20.6	189.6
		29.0	68.4
		39.4	21.4
		48.1	10.7
		71.1	2.6
Sage[a]	18.6	11.7	729.6
(*Salvia*)		20.2	184.8
		30.7	55.2
		40.9	19.2
		50.7	9.5
White clover[b]	13.7	25.0	420
(*Trifolium repens*)	14.2		269
	15.5		138
	17.1		69.0
	18.2		48.1
	19.1		34.9
	20.2		20.4
	21.5		13.6
Sage[b]	16.5	25	115
Sweet clover[b]	16.5	25	87.5
White clover[b]	16.5	25	94.0

[a] Data of Munro (1943).

[b] Interpolated from Munro's data.

TABLE XIV

RELATIVE FLOW OF HONEY IN PIPES[a]

Pipe diameter (inside)	Temperature		
	82°F (28°C)	102°F (39°C)	122°F (50°C)
¾ in. (19 mm)	149	400	1125
1 in. (25 mm)	367	973	2353
1¼ in (31 mm)	729	1895	5000
1½ in. (38 mm)	1263	2609	6792

[a] Rate of flow (in pounds per hour) through 4-inch (10-cm) length of pipe with 4-inch head. Data of MacDonald (1963).

sizes, the rate of flow increases equally with each of the two temperature increments. The importance of pipe diameter in moving honey at the lower temperatures is shown by the eightfold increase in flow obtained when the cross-section area of the pipe is increased four times. This effect declines as the viscosity decreases with increasing temperature.

Most honeys are Newtonian liquids but some have been reported to have non-Newtonian properties. Pryce-Jones (1953) has examined the rheology of heather honey, which is so thixotropic that it cannot be removed from the comb by a centrifugal extractor unless the gel-sol transformation is effected by applied vibrating rods. This property is ascribed to the properties of the proteins; if isolated heather honey protein is added to clover-honey, it exhibits thixotropic behavior. Manuka (*Leptospermium scoparium*) honey from New Zealand and Karvi (*Carvia callosa*) from India (Deodikar *et al.*, 1957) are markedly thixotropic. Pryce-Jones (1952) also reported that *Opuntia* honey from Nigeria and several *Eucalyptus* types exhibited dilatancy, which he ascribed to the presence of a high-molecular dextran.

B. THERMAL PROPERTIES

Relatively little data are available on the physical properties of honey with respect to heat, even though honey can easily be damaged by its improper application. Processing equipment design has generally been based on data from sugar processing.

The specific heat of honey at 17.4% moisture was reported by Helvey (1954) to be 0.54 cal/gm/°C at 20°C with a temperature coefficient of 0.02 cal/°C. He also measured specific heat of honey solutions. Townsend (1954b) has described McNaughton's determination of specific heat. He used a considerably larger sample and obtained somewhat higher results, as seen in Table XV.

TABLE XV

SPECIFIC HEAT OF HONEY[a]

Moisture content (%)	Specific heat
20.4	0.60
19.8	0.62
18.8	0.64
17.6	0.62
15.8	0.60
14.5	0.56
Coarsely granulated	0.64
Finely granulated	0.73

[a] Data of MacNaughton (Townsend, 1954b).

Basic data necessary in designing a heat exchanger for honey processing have been obtained by Detroy (1966). Using a concentric-tube exchanger, the surface conductance or film coefficient for honey was determined at flow rates of 700–975 lb/hour and two temperature ranges of interest in processing, preheater (65°–68°C) and flash heater (85°–88°C). Figure 21 shows the values calculated from these data for a range of honey flow velocities. In this work honey was in laminar flow, water in turbulent flow. Detroy used Helvey's value for specific heat of 0.54. He pointed out the desirability of experimental verification of his values

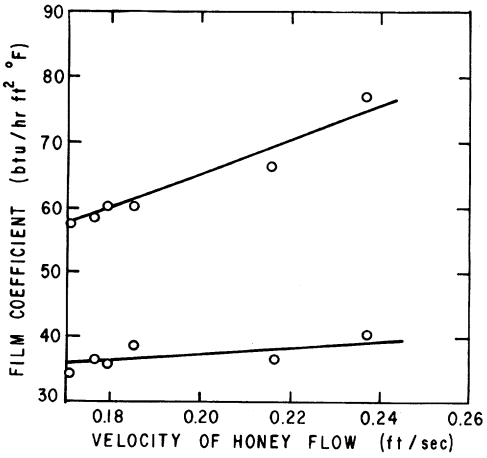
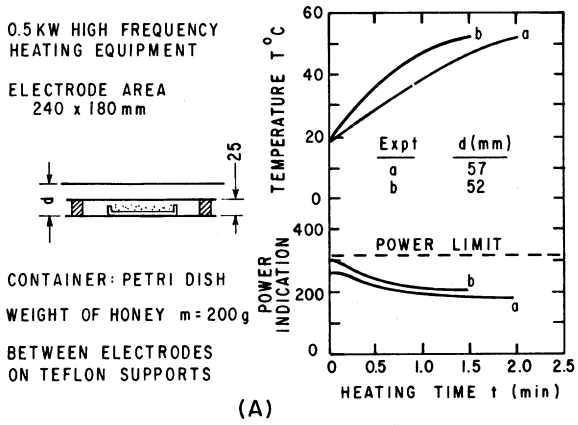


FIG. 21. Change of film coefficient with velocity of honey flow in the honey-to-water temperature difference range of each heating circuit. Upper line, flash heater water circuit; $Y = 10.6 + 272x$; $S_{y,x} = 1.68$. Lower line, preheater water circuit; $y = 26.51 + 53.3x$; $S_{x,y} = 1.7$. (From Detroy, 1966.)

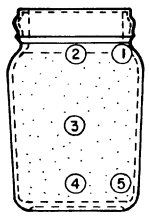
under accurately controlled conditions, since the possibility for experimental error is considerable.

The heat sensitivity and relatively low heat conductance of honey have encouraged examination of high-frequency heating of honey. Lackett and Wilson (1971) used a kitchen-type microwave oven operating at 2450 mHz to heat and liquify completely granulated honey in 1-lb jars. With the metallic caps removed, heating was rapid but not uniform; when a temperature of 60°C was reached in the center of the jar, a damaging 98°C was reached at the top. Difficulty was encountered in heating larger jars (2½ lb) without boiling the surface layers.

Bergel and Stuwe (1972) have proposed the use of dielectric heating for honey processing. They have estimated from small-scale heating experiments that a 25-kW dielectric heating installation would be required to heat 1000 kg per hour from 30° to 55°C. The frequency was not specified. Normal range for dielectric heating is 2–100 mHz. The arrangement and results of the small-scale tests are



(A)



(B)

Expt.	d (mm)	t (min)	T° (°C)	T°(°C) at ind. pt. ①	②	③	④	⑤
12a	135	1.0	20	51	46	50	44	42
12b	127	1.0	20	55	58	55	48	46
13b	138	1.5	20	59	59	56	51	50
13c	138	1.5	20	55	50	57	51	51
14b	140	2.0	20	67	70	63	57	55
14c	145	2.0	20	57	56	60	56	55
15a	150	2.5	20	63	60	63	59	60
15b	160	2.5	20	54	53	59	57	56
15c	175	2.5	20	52	51	56	52	51

FIG. 22. (A) Dielectric heating of honey in a glass dish. (From Bergel and Stuwe, 1972.) (B) Temperature variation in high-frequency heating of honey in a jar. (From Bergel and Stuwe, 1972.)

shown in Fig. 22A. The authors point out that, as seen in the figure, the power absorption decreases with increasing temperature, i.e., the dielectric loss value for honey decreases with increasing temperature. This automatically provides temperature equalization. In Fig. 22B is seen the results of heating honey in a commercial jar (500 gm) with metal cap. Under the proper conditions the jar of honey is heated to 59°–63°C in 2½ minutes. Overheating induced boiling in a ring under the cap. Analysis of all honey samples in the latter experiment showed no change in diastase value or HMF content. Samples remained liquid for at least 6 months. By heating 1 kg in two jars in the 2-kW chamber at full power, a linear heating rate of 32°C/minute was found between 20° and 60°C. From this a high frequency power requirement of 25 kW was calculated for heating 1000 kg/hour from 30° to 55°C.

C. HYGROSCOPICITY

1. Equilibrium Relative Humidity

The ripening of nectar to honey by the bee includes its repeated exposure in a thin film to warm air. The solids content reached is a function of the extent of moisture saturation of the air in the hive, which is related to temperature and to the external air conditions. Nearly all honey contains less glucose than fructose, the more hygroscopic carbohydrate, and is remarkably hygroscopic for a natural material. As seen in Table XVI, honey in its normal moisture range of 16.8–18.3% is in equilibirum with air at 55–60% RH. In general, attention must be given to hygroscopicity in handling and processing, since, as Martin (1958) has

TABLE XVI

APPROXIMATE EQUILIBRIUM BETWEEN RELATIVE HUMIDITY OF AIR AND THE WATER CONTENT OF A CLOVER HONEY[a]

Relative humidity (%)	Water content (%)
50	15.9
55	16.8
60	18.3
65	20.9
70	24.2
75	28.3
80	33.1

[a] Interpolated from the data of Martin (1958).

shown, moisture from the air diffuses only slowly into the mass, so that aerobic yeast growth is encouraged at the surface. For example, Martin (1958) showed that a honey sample at 22.5% moisture exposed to air at 86% RH for 7 days had 26% moisture in the surface layer; 2 cm below no change was found.

2. *Comparison with Other Carbohydrates*

Relatively few data are available to provide for comparison of commercially available carbohydrates. Table XVII summarizes values for honey, invert syrup, fructose syrup, and commercial glucose at 20% moisture. Uncertainty among values of different investigators makes it difficult to determine if real differences exist among honey, invert syrup, and fructose syrup. Conventional corn syrup is definitely less hygroscopic. Data are not available for high fructose corn syrup.

D. CRYSTALLIZATION

1. *Glucose*

a. Cause and Prediction. As noted earlier, the stable form of most extracted honey is a matrix of glucose hydrate crystals in a syrup. This is due to a considerable extent to the lower storage temperature to which the honey is exposed after removal from the bee colony. Nearly all honey is supersaturated with respect to glucose except for a few nongranulating types that are relatively low in glucose, such as tupelo and sage. Crystallization of glucose from honey while in the

TABLE XVII

EQUILIBRIUM RELATIVE HUMIDITY OF VARIOUS CARBOHYDRATES AT 20% MOISTURE[a]

Material	E.R.H.	Reference
Honey	63.5%[b]	Lothrop (1937)
	63.2	Martin (1958)
Invert sirup	67	Lothrop (1937)
	67.5	Dittmar (1935)
	57.5	Money and Born (1951)
Levulose sirup	63.5	Money and Born (1951)
	61.3	Lothrop (1937)
Commercial glucose	75	Lothrop (1937)
	72	Money and Born (1951)

[a] Most values interpolated from original data.
[b] Average of five samples.

comb, though relatively rare, may be encountered with such honey as dandelion, blue curls, and ivy. It is likely that the extraction process encourages subsequent granulation by introducing fine glucose crystals from equipment, from the air of the extracting plant, and from containers. Natural crystallization, before heating, is usually fine grained, reflecting the presence of myriads of fine seed crystals and initiators such as dust, pollen, and fine air bubbles. After honey is heated and/or filtered, seed crystals are no longer present, and when crystallization finally takes place it is usually coarse grained and slow. Proper heating and processing will delay granulation for many months.

Two general approaches to predicting granulation tendency of honey have been made: study of model systems and empirical correlation of various parameters with observed behavior. Examples of the former are the work of Jackson and Silsbee of the U.S. Bureau of Standards, Lothrop of the U.S. Department of Agriculture, and Kelly of the University of Tasmania. Jackson and Silsbee (1924) examined several systems at 30°C and discussed the glucose-fructose-water system with reference to honey. In the presence of solid glucose hydrate, solubility of glucose decreased from 54.6% without fructose to 32.5% at 39.4% fructose. Their conclusion that all honey is supersaturated with respect to glucose (even never-granulating tupelo honey) was based on inadequate analytical procedures then used for honey, which overestimated glucose; in addition, their data did not extend to the higher fructose concentrations found in some honey. Lothrop (1943), in an unpublished thesis, extended their data to higher fructose concentrations. He found an abrupt increase in dextrose solubility at a fructose concentration of about 150 gm in 100 gm water. The solid phase in the region of higher solubility was anhydrous glucose. Identification was by crystal form. Lothrop felt that the increased solubility was not related to the α-β equilibrium, but rather to the extent of hydration of the glucose in solution, and concluded that this accounted for the failure of certain honeys to crystallize. Lothrop's data, replotted on a ternary diagram, are shown in Fig. 23, since they were never published. Kelly (1954), without knowledge of Lothrop's work, published the complete diagram for the system at 30°C. He also noted an area in which anhydrous glucose is the solid phase (Fig. 24) and an invariant point at which both forms are in equilibrium. He suggested that in solutions saturated with fructose, the transition temperature of the monohydrate was reduced from >50°C to <30°C. He noted that published analyses of honey relate to the area at which anhydrous glucose is in the solid phase at 30°C. However, honey crystallizes only below 30°C, below the transition temperature, so that only the hydrate crystallizes. Villumstad (1952) has described the simultaneous presence of both plates and needles of glucose in honey, without speculation on the reason.

Kelly (1957) suggested using the quaternary glucose-fructose-sucrose-water diagram (Kelly, 1955) in an effort to predict granulation. As a first approximation, all disaccharides were considered as sucrose, and a honey composition fell

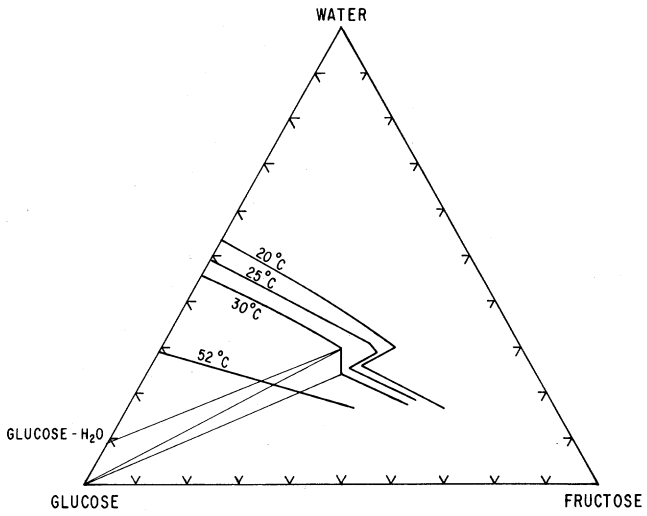


FIG. 23. Effect of temperature on the fructose-glucose-water system. (From Lothrop, 1943.)

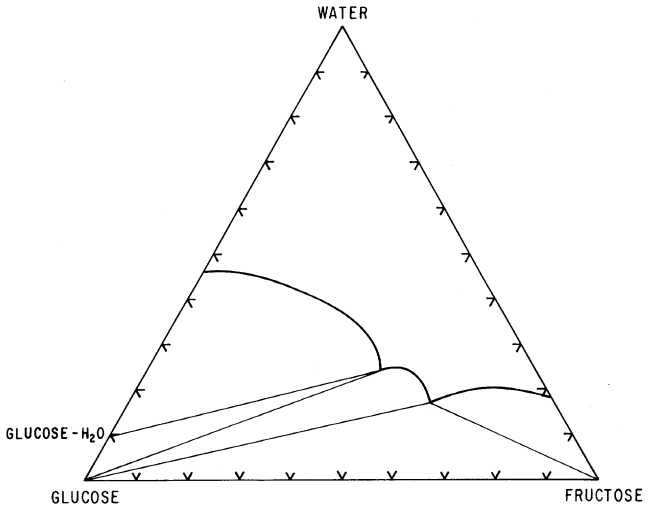


FIG. 24. The system water-glucose-fructose at 30°C. (From Kelly, 1954.)

into the area in which the solid glucose is anhydrous at 30°C, but hydrated at some point below 30°C. An additional complication may be the finding of Dean (1976) of a phase in the glucose-water system thought to be β-D-glucose monohydrate metastable at 38°–50°C, transforming to stable α-D-glucose monohydrate at 32°–38°C. The metastable phase is apparently orthorhomic, as are both anhydrous forms. A further problem in applying model systems is the presence of a variety of other sugars at >1% of the total.

Knowledge of granulating tendency would be useful in selecting honey for both liquid and semisolid pack. Earliest attention was given to the ratio of fructose to glucose for prediction, but difficulty was encountered (Jackson and Silsbee, 1924) because of inadequate anlytical methods for these sugars on mixture. White *et al.* (1962) reviewed the literature and calculated several of the proposed indices for the 490 honeys they analyzed. The ratio *D/W,* proposed by Austin (1958), showed a highly significant relationship with granulating tendency (as defined) equivalent to the *D-W/L* of Jackson and Silsbee (1924). White *et al.,* considering that the latter required more analytical work, suggested that the *D/W* ratio be used; values of $\leqq 1.7$ seemed to be generally associated with nongranulating honeys; values $\geqq 2.1$ would predict rapid granulation to a solid.

Codounis (1962) has suggested that the relationship *B-D/D* (B = Brix, D = glucose) provides a better index. Hadorn and Zürcher (1974) found only a loose association between D/W and subsequent crystallization of honey. The calculations of White *et al.* (1962) were based on observation of the extent of granulation of samples after 6 months of room temperature storage following liquefaction. A more useful index may have resulted had the samples been seeded with glucose hydrate after cooling.

b. Controlled Crystallization. Since the texture of the semisolid pack depends upon a three-dimensional interlaced network of dextrose hydrate crystals in a liquid phase, it will not return to the initial hardness after stirring or softening by heat or improper storage. The initial texture is influenced by moisture and dextrose content of the honey and by the amount and quality of the seed material. Best (smoothest) texture results from growth of a network of interlaced fine crystals, induced by large numbers of very fine crystals and fragments in the seed material.

The temperature of the honey at the addition of the seed must be low enough to avoid destroying the smallest crystal particles in the seed. The addition of seed at 14°C with continued blending at that temperature however does not seem to lead to the most desirable texture because too much of the crystallization takes place too early to permit an adequately interlaced network of crystals to form in the retail container.

This product is not commonly packed in clear glass, but rather in waxed paperboard or plastic tubs or jars or in opaque glass jars. The reason appears to be

that changes at the container-honey interface can give the appearance of spoilage, although the product is in good condition. This is an uneven separation of the contents from the wall of the container, sometimes combined with a white irregular patchy appearance termed "frosting." Thomson (1938), in a detailed study of this, concluded that it resulted from the presence of air bubbles in the honey before setting, together with a contraction of volume accompanying granulation. Crystals in frosted areas were found to be smaller than in other areas and consequently appeared lighter in color. The volume contraction was found to be 0.500 cc per 100 gm, or about 0.60% *V/V*.

No consideration was given by Thomson to avoiding or eliminating the defect. Green (1951), without reference to Thomson, ascribed frosting to the presence of dissolved air and tiny air bubbles in the honey and their segregation by rejection from the crystal. He ascribed no relevance to the composition of the container wall and proposed no remedies. Gonnet and Lavie (1963) studied the adhesion of crystallized honey to container walls. They ascribed separation to thermal shock (rapid temperature drop) and also to continued storage at 14°C after setting, with separation occurring in the third week of such storage. They eliminated it by exposure to 30°C, but noted it recurred after sharp cooling. The color change was ascribed to desiccation of the exposed glucose crystals. They examined several treatments of the glass container walls to eliminate this without marked success. McDonald (1964) also studied the factors affecting the formation of voids (i.e. space between container and product) and recommended prompt removal from the 14°C crystallizing temperature after 5 days to 20°C or higher temperatures, followed by conditioning. A conditioning for 2 days at 35°C of the particular honey used prevented subsequent void formation regardless of subsequent lower storage temperature; McDonald indicated that lower temperatures might be preferable for honey of higher moisture content, since considerable softening was evident after conditioning. Guilbault (1965) included this problem in his study of quality factors in recrystallized honey packs. He recommended conditioning in opaque packs after the 4–5 days 14°C crystallizing step by storage 7–14 days at 25°–27°C followed by holding 10 days or till shipped at 14°C. For glass packs, holding for not over 5 days at 30°C would prevent subsequent frosting. Other recommendations included control of seed quality by making crystal counts, since a 100% variation can occur in this parameter, which affects product quality.

2. *Melezitose*

Other than glucose, melezitose is the only sugar known to crystallize from honey, or more correctly, from honeydew honey. It is relatively rare to encounter sufficiently high concentrations of melezitose for crystallization. Such crystallization usually takes place in the comb. Hudson and Sherwood (1920) described

several instances where this occurred, finding 10–25% melezitose in the honeydew honeys they described.

VI. STORAGE OF HONEY

A. EFFECTS OF TIME AND TEMPERATURE

It is not surprising that honey is susceptible to physical and chemical change during storage, considering its nature as a highly concentrated, somewhat acid solution of fructose and glucose. The changes caused by heating also take place at any temperature above about 5°C.

1. Color Development

Depending upon the composition (fructose, moisture, acidity) honey darkens during storage and heat processing. Considerable variation among samples has been observed. Noting that addition of formaldehyde prevented darkening of honey on storage, Ramsey and Milum (1933) proposed that most of the darkening resulted from the Maillard reaction between amino acids and reducing sugars. The most extensive study if that of Milum (1948). He concluded that darkening during storage is dependent in part upon the amount of previous darkening; thus discoloration during processing tends to reduce the subsequent rate. Table XVIII shows a summary of Milum's data obtained by replotting and interpolation. The values should be regarded as approximations only. Smith (1967) stored several Australian commercial-type honeys at temperatures between 43° and 80°C, all

TABLE XVIII

APPROXIMATE RATE OF HONEY DARKENING IN STORAGE[a]

Temperature of storage		Darkening in mm Pfund/month		
°F	°C	Original color < 34 mm	Original color 34–50 mm	Original color > 50 mm
50	10.0	0.024	0.024	0.024
60	15.6	0.08	0.125	0.10
70	21.1	0.27	0.70	0.40
80	26.7	0.90	4.0	1.5
90	32.2	3.0	7.7	5.0
100	37.8	10.0	14.0	11.0

[a] Calculated from data of Milum (1948).

above those given in Table XVI. One honey (*Dryandra*) was more unstable than the others. Smith found for the remaining five that the times required at various temperatures to produce 10 mm darkening (Pfund) were of the same magnitude as those calculated by White *et al.* (1964) to produce 3 mg HMF/100 gm honey.

Wootton *et al.* (1976a) examined the changes induced by storage of six Australian honeys at 50°C for 44 days, analyzing for color, acidity, total nitrogen, sugars, and free amino acids. Rate of increase in color varied markedly; the least stable required approximately 5 days to increase 20 mm in color, and the most stable, about 16 days. This is a wider range than found by Smith. The retarding effect of added sulfite indicated the Maillard reaction to play a major role; ascorbic acid addition had no effect, eliminating an oxidative mechanism.

Changes in carbohydrates (Wootton *et al.*, 1976b) differed from those reported by White *et al.* (1962) and Kalimi and Sohonie (1964), but all experimental factors involved differed. Amino acid content decreased markedly for five honeys (26–83% decrease), and apparently increased (6%) for one, tea-tree (*Leptospermum scoparum*). Most of the quantitative decrease was loss of proline, which represented approximately 80% of the free amino acids, except for tea tree (about 30%). Increases were found in a few (1–3) amino acids, losses in all others; again tea tree was the exception, showing an increase in total amino acids, including 7 of the 15 present. A major increase in phenylalanine was recorded for alfalfa and tea-tree honeys. The greatest decrease reported would remove only about 0.16% of monosaccharide from the honey, less than the analytical error in the sugar analysis. Wootton *et al.* ascribed the increases to protein breakdown.

2. *Hydroxymethylfurfural*

Several colorimetric tests were devised many years ago to indicate the addition of acid-inverted syrup to honey. These tests, the resorcinol (Fiehe) test, and the aniline (Feder) test have been intensively studied and modified. Considerable early controversy was concerned with the interpretation of the colors produced. These reagents were known to be reacting with hydroxymethylfurfural (HMF), which is formed from fructose by action of acid and heat. Invert sugar prepared with acid contains variable amounts of HMF, depending on the conditions used. The minimum amount of added invert sugar detectable by these tests thus depends on their sensitivity and the characteristics of the invert syrup used. Much controversy in the literature revolved about this because the color tests responded differently for different investigators, thus in effect using differing standards for judging adulteration by invert sugar.

It was long ago recognized (Browne, 1908) that honey if heated sufficiently would give a positive test. Such heating was said to destroy the flavor characteristics. Nothing is to be gained by reviewing earlier studies, of which there

were many, which attempted to show that the Fiehe and Feder tests were, in fact, suitable to distinguish between heated and adulterated honey. For example, these included studies (Shannon, 1916; Sherwood, 1924; Greenleaf and Browne, 1929) in which collaborative testing was done, and two papers concluding that the color tests indeed were useful (Lampitt *et al.*, 1929; Gautier *et al.*, 1961). de Boer (1934) suggested that extended ordinary storage could also lead to accumulation of enough HMF for a positive test, the time required being a function of the storage temperature.

The publication by Winkler (1955) of two quantitative methods for HMF in honey provided the means for European countries to extend their examinations of imported honey to include the HMF levels of honey, as well as diastase content. These countries had for many years insisted that honey sold for direct consumption meet minimum standards for diastase to assure that it had not been "denatured" by overheating. They ascribe rather obscure health-giving properties to honey which they feel are vitiated by heating. Enforcement of these rules has provided a considerable volume of analytical data on honey from the major exporting countries. When the quantitative HMF method became available, limiting values for HMF content of honey were established in addition to diastase values. An extensive collection of analyses of imported honey is summarized in the paper of Duisberg and Hadorn (1966), where HMF analyses of 1554 imported samples of commercial honey received between 1960 and 1966 by laboratories in Switzerland and Germany are given. Figure 25 shows the results.

Using Winkler's methods for quantitation of HMF in honey, Schade *et al.*

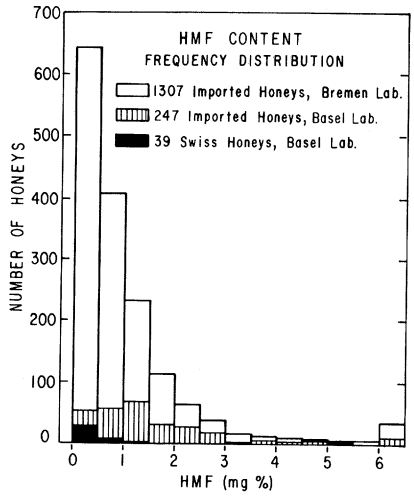


FIG. 25. Frequency distribution of HMF content of honey. (From Duisberg and Hadorn, 1966.)

(1958) demonstrated variability among honeys, of the effects of storage and heating, and of the compositional factors influencing HMF formation, and reported the rate to correlate directly with moisture content and with initial HMF content. Other unknown factors also affected rates. Lampitt *et al.* (1929) had earlier confirmed the positive effect of the acidity of honey in the formation of HMF; Hadorn *et al.* (1962) ascribed the lower rate of HMF formation in heated Swiss honeys to their higher pH (4.5–5.0) in contrast to that of most other honey (pH 3.8). Several groups of workers have reported the effect on HMF content of various heat treatments of honey. The temperature exposures ranged from those normally used in honey processing to deliberately excessive treatments, in terms of deleterious effect on general organoleptic qualities.

Schade *et al.* (1958) showed HMF to increase in four samples during storage 13–15 months at 20°C (68°F); in one case an increase of 3.3 mg% was recorded. Three alfalfa honeys accumulated HMF at higher temperatures as shown in Fig. 26. Hadorn and Kovacs (1960) reported the effect of holding several types of imported honey at 50°C (122°F). Their results are summarized in Table XIX. Most investigators reported data from small-scale laboratory tests. An exception is the work of Hadorn and Zürcher (1962), who followed HMF content in 300-kg (660-lb) drums as they were taken through the procedure normally used in Switzerland to liquefy the contents, holding in a room at 48°C. The honey reached 48°C in 24 hours. At 120 hours, HMF content in three drums had increased from 1.2 to 2.2, 2.7, and 2.4 mg/100 gm.

White *et al.* (1964) subjected three honey samples to storage at seven temperatures ranging from −20° to 60°C (−40° to 140°F) and analyzed them for HMF

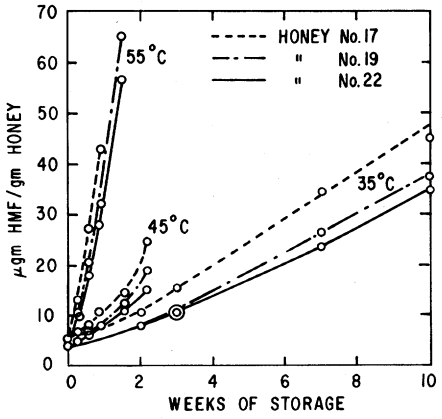


FIG. 26. Comparison of the rate of formation of HMF in three alfalfa honeys during storage at elevated temperatures. From Schade *et al.*, 1958. Reprinted from Food Technology/*Food Research* **23,** 446–463, 1958. Copyright © by the Institute of Food Technologists.

TABLE XIX

HYDROXYMETHYLFURFURAL CONTENT OF HONEY HELD AT 50°C[a,b]

Source	Initial value	100 hr	300 hr
Guatemala	2.5	5	16
Central America	0.6	2	8
California	1.6	4.3	8
Mexico	0.1	0.8	26

[a] Interpolated from graph of Hadorn and Kovacs (1960).
[b] In mg/100 gm of honey.

content. Figure 27 shows the approximate time required for a honey to accumulate 4 and 20 mg HMF/100 gm.

The effective use of HMF levels to demonstrate addition of invert sugar to honey requires that a maximum value be established representing the combined effects of commercial storage and processing so that genuine, albeit storage or heat-abused, honey not be discriminated against. Variability in the response of honey to heat compounds the difficulty of setting an equitable level. In any event, since honey may, due to economic conditions, be stored a year or more in

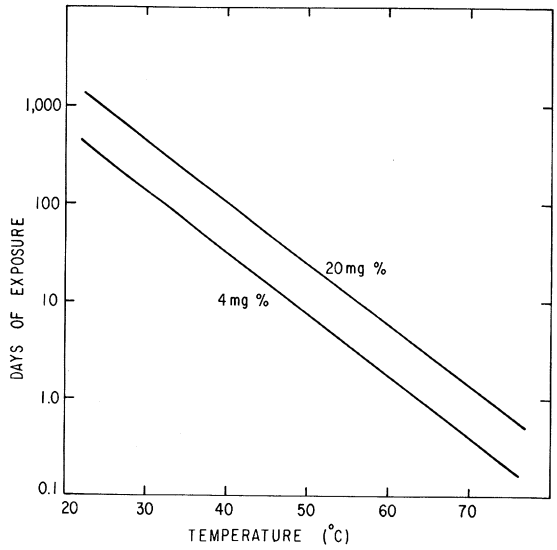


FIG. 27. Heat exposure required to develop indicated amount of HMF in honey. (Calculated from data of White *et al.*, 1964.)

high ambient temperatures at tropical places of production, a history of storage of suspected samples may prevent unjustified allegations of adulteration.

3. *Flavor Changes*

As honey is heated or stored for several months at temperatures common to much of the United States, the more delicate aspects of flavor and aroma may change. These changes are relatively minor; to detect them a sample must be compared with a control kept at freezer temperatures. It is easily possible to damage flavor by excessive heating; heating that causes darkening will certainly have a deleterious effect on flavor. Fresh honey in the comb, a delicacy of yesteryear, has the maximum of volatile "top notes" and desirable flavor quality. Present-day closed-system high-temperature short-time processing does provide a better flavored product than do the batch processes that were previously used and are still used by some smaller operators.

4. *Enzyme Inactivation*

The effect of storage on enzyme inactivation is of importance largely in honey intended for export to countries with minimum limits for diastase (and in some cases, invertase). Difficulty has been encountered with rejection of United States shipments by European countries over many years.

Many papers describe the effect of elevated temperatures on enzyme activity, particularly diastase, in honey, but it remained for Schade *et al.* (1958) by applying their quantitative procedure for honey diastase to record the effect of storage at 20°C on diastase. The loss which they described as "slight, but not significant in most cases" can be calculated at about 10% in 13–15 months. Later White *et al.* (1964) emphasized that the changes are relatively predictable over the temperature range of 10°–80°C; Table XX shows the half-life of honey diastase and invertase over this range, based on their data.

5. *Carbohydrate Composition*

The most obvious change in the sugar of honey takes place during ripening, with the inversion of sucrose and the production of transglycosylation sugars, which persist in the ripened product. Enzymic inversion continues in full-density honey at a greatly reduced rate and can contribute to error in the analysis of sucrose. The most striking evidence is seen in certain floral types that on occasion produce so heavily in relatively warm, dry weather that the honey reaches full density while the sucrose content is still 10% or more. This may lead to legal difficulties, since maximum permitted values for sucrose can be as low as 5%. Unless the honey is heated, the sucrose content can be expected to decline to

TABLE XX

CALCULATED HALF-LIVES OF HONEY ENZYMES[a]

Temperature		Half-life	
°C	°F	Diastase	Invertase
10	50	12,600 days	9,600 days
20	68	1,480 days	820 days
25	77	540 days	250 days
32.2	90	126 days	48 days
35	95	78 days	28 days
40	104	31 days	9.6 days
50	122	5.4 days	1.3 days
62.4	145	16 hr	3 hr
71	160	4.5 hr	40 min
80	176	1.2 hr	8.6 min

[a] From White *et al.* (1963a).

legal levels, although the time required is variable. Smith (1965) in Australia described a crop of honey from *Banksia menziesii* which fell from 8–12% sucrose to about half that in a year's storage. The acacia (locust, *Robinia pseudoacacia*) honey flow is at times of this nature; Borus *et al.* (1966) reported an instance in which locust honey of 9.6–12.7% sucrose fell to 1.7–4.3% in a year's storage. In general, the nature of the honey flow, as described above, results in lower-than-normal levels of bee-added enzymes. Citrus honey is also one of those the Germans call "naturbelassen," being generally low in enzymes for this reason. Three 1976 citrus honey samples with sucrose contents immediately after extraction of 5.3, 7.1, and 9.3% fell during 12 weeks room temperature storage to 2.9, 3.1, and 2.2%, respectively (White, 1976).

Changes in other carbohydrates during storage are not as obvious. Täufel and Müller (1953), finding minor sugars in honey by paper chromatography, suggested that they might arise from acid or enzyme conversion of the major honey sugars. Later (Täufel and Müller, 1957) they concluded, using conventional analyses and paper chromatography, that significant changes do not occur on storage. Using more appropriate analytical procedures and statistical treatment, White *et al.* (1961) examined the effect of storage of honey on carbohydrate composition. In this work, honey stored at −20°C was compared with aliquots held up to 2 years at room temperature, with and without heating 30 minutes at 55°C for pasteurization, without excessive enzyme inactivation. The storage caused an increase of 69% in reducing disaccharides, a slight increase in sucrose and higher sugars at the expense of glucose and fructose, which decreased 13 and 5.5%, respectively. It is likely that this decrease in glucose is a

major cause of texture loss and partial liquefaction of finely granulated honey during long-term storage.

These changes would appear to be caused by two mechanisms: enzyme activity and acid reversion. At the low water concentration in ripe honey, the formation of disaccharides by slow α-glucosidase action should be favored over the accumulation of free hexoses by transfer to water as the acceptor. In concentrated solutions of monosaccharides in the presence of acids, appreciable reversion to disaccharides and higher sugars takes place (Pigman and Goepp, 1948).

B. FERMENTATION

The development of commercial honey production during early decades of this century encountered major difficulties, particularly in Canada, with fermentation. Osmophilic yeasts, which can ferment honey even at its low water activity, are nearly ubiquitous on the bodies of bees, in nectar, soil in apiaries, and extracting and storage areas. Even though a honey be in the "safe" area (Table V) the subsequent granulation will enrich the liquid phase in water and increase the risk. Many investigators in the northern United States and Canada studied this problem between 1928 and 1932, with the most definitive work that of Lochhead and his co-workers (Lochhead and Heron, 1929; Lochhead and Farrell, 1930a,b, 1931a,b; Lochhead, 1933; Marvin, 1928, 1930; Wilson and Marvin, 1929, 1931, 1932; Marvin *et al.*, 1931). Table XXI lists the yeasts isolated from honey. Martin (1958), in his work on hygroscopicity, examined factors leading to yeast growth at the surface and also in the depth of the container. He found that, when

TABLE XXI

YEASTS ISOLATED FROM HONEY

Type	Reference
Nematospora ashbya gossypii	Aoyagi and Oryu (1968)
Saccharomyces bisporus	Aoyagi and Oryu (1968)
Saccharomyces torulosus	Aoyagi and Oryu (1968)
Schizosaccharomyces octosporus	Lochhead and Farrell (1931b)
Schwanniomyces occidentilis	Aoyagi and Oryu (1968)
Torula mellis	Fabian and Quinet (1928)
Zygosaccharomyces spp. (2)	Nussbaumer (1910)
Zygosaccharomyces barkeri	Lochhead and Heron (1929)
Zygosaccharomyces japonicus	Aoyagi and Oryu (1968)
Zygosaccharomyces mellis	Fabian and Quinet (1928)
Zygosaccharomyces mellis acidi	Richter (1912)
Zygosaccharomyces nussbaumeri	Lochhead and Heron (1929)
Zygosaccharomyces priorianus	Fabian and Quinet (1928)
Zygosaccharomyces richteri	Lochhead and Heron (1929)

surface moisture increased above about 22%, yeast count increased massively at the surface; although from 2 cm down, counts remained stable. Further handling then can distribute the inoculum throughout the mass with subsequent anaerobic fermentation.

C. RECOMMENDED STORAGE FOR HONEY

Problems to be considered in storage of honey are fermentation, granulation, discoloration, flavor damage, and, if intended for export, destruction of enzymes and production of HMF.

The only condition in which all dangers and changes are eliminated is in freezer storage. Since this is not a practical procedure, some compromise is to be expected, depending upon the type and intended use of the honey.

In any case, honey must be protected from atmospheric moisture. Cold (i.e., below 10°C) storage has been recommended for unprocessed honey to prevent fermentation (Marvin, 1928). Fermentation generally does not take place in unheated warehouse storage in northern winters, but it can be expected when temperatures become favorable, since most honeys will have granulated by then. Pasteurized honey, though not liable to ferment, will granulate (coarsely) if held in fluctuating temperatures between 11° and 15°C; this will necessitate further processing. Processed honey is best stored between 18° and 24°C; short-term exposure to higher temperature is permissible. Since heat damage is additive, care must be taken to limit heat exposure as much as possible: 10 days at 32°C are equivalent to 100–120 days at 21°C (White *et al.*, (1963b). Reduction of storage temperature by 6°–8°C will reduce rate of deterioration to ⅓–⅙ of that at the higher temperature.

VII. NUTRITIVE VALUE

A. AS A CARBOHYDRATE

There is probably no area in which scientific opinion clashes with folklore more than in the nutritive (and medical) aspects of honey. Many articles in the lay or trade press over the years in all parts of the world attest to the superiority of honey as a nutrient. The subject was recently reviewed in the book edited by Crane (1975a) in a chapter on the biological properties of honey. There, six collaborators agreed that "the time had come to make a realistic appraisal of the position and to clear away some of the misconceptions that are published from time to time." After noting that over 2000 papers and articles have been published on the subject and referring to several books that have appeared since the second World War, several aspects are discussed that appear to be relatively well founded, including nutritive value. Honey is a nutritive sweetener, with prop-

erties arising from its high content of glucose and especially of fructose and its variable content of trace minerals. As noted elsewhere, the vitamin content has no nutritional significance.

Haydak (1936) described a study in which he continued normal work for a 12-week period on a diet consisting solely of milk and honey. No subjective or clinical problems were noted, except a need for ascorbic acid supplementation. Later (Haydak *et al.*, 1944), five adults alternated 1-week test periods with 1-week normal diet for 4 weeks. The test diet, milk and honey, was supplemented with thiamine, ascorbic acid, and iodide. No effect on normal health and activity was noted. Haydak (1955) reviewed the nutritional aspects in general.

The rapid absorption of honey monosaccharides and the slower metabolism of the fructose content appear to be the basis of its popularity as a source of quick energy for athletes, scuba divers, and mountain climbers. Townsend (1954a) reviewed this aspect.

1. Infant and Geriatric Diet

A considerable literature, largely European, has accumulated in the past 50 years on the value of honey supplementation of milk in infant feeding. For details the reader is directed to the reviews of Haydak (1955) and White (1975b). The facile absorption of the monosaccharides, improved weight gain, relief from constipation, decrease of diarrhea, and good tolerance by infants at special risk is cited. Improved calcium retention (Knott *et al.*, 1941) and utility in feeding prematures (Vignec and Julia, 1954) are also cited. Most of the articles conclude that honey should have a wider use in infant feeding.

At the other end of the human experience, honey appears to have some particular utility in geriatric feeding. Albanese *et al.* (1954) pointed out that utilization of glucose is markedly decreased with aging, while that of fructose is only slightly affected. Results of his experiments suggest that levulose or levulose-containing products are sugars of choice for the aged in that they may provide a ready source of energy and an optimal protein-sparing effect. In a later study Albanese *et al.* (1968) reported that glucose tolerance is not significantly altered by age in healthy subjects, but a greater loss of tolerance to glucose than to honey was seen in patients recovering from strokes. Similar differences prevailed in patients with diabetes, with hemiplegia complicated by diabetes, and in those recovering from coronary occlusion. Distinct differences in the metabolism of glucose and fructose have been reviewed recently (Pawan, 1973).

2. Honey and Diabetes

Beekeeping and lay publications at times contain suggestions that diabetics can use honey without incident. This is nonsense, since honey contains a considera-

ble proportion of glucose. Because on the weight basis, honey (at 80% solids) is about as sweet as sugar and provides an average of 31% as glucose, compared with sugar's 52%, some advantage in "sweetening power" is theoretically available to the stabilized diabetic. Selection of tupelo honey, averaging about 25% glucose, would approximately double the "sweetness" intake without increasing glucose. It must be emphasized, however, that any such substitution be undertaken only upon the advice of the physician.

B. MINERALS AND VITAMINS

As already noted, honey does contain measurable amounts of several vitamins and quite variable levels of a number of minerals. The real nutritional significance of these may be assessed by examination of Table XXII, which shows order of magnitude for the more important nutrients in relation to the United States minimum daily requirements.

C. FOLKLORE

Space is not available for a discussion of the folklore of honey, which dates back about 5000 years. The interested reader is referred to the excellent review by Crane, "History of Honey" (1975c), and to the less scholarly "Honey and Health" by Beck (1938).

VIII. USES

A. FOOD

Most of the honey sold for food is used directly as table sweetener or spread. The most significant indirect uses are in baking, cereal, and confectionery. Use in baking has decreased in recent years because of the run-up in price and the introduction of fructose-containing syrups that approach the functional values of honey. Unduplicated, however, are the flavor advantages conferred by honey and the freedom to use the word "honey" in advertising and promotion, which carries a definite connotation of quality and "old-fashioned goodness" not conferred by any other sweetener.

Over the years articles have appeared in the baking trade press describing the use of honey in various products. Griffith (1934a,b,c) produced information and recipes for crullers, sweet rolls, rye and white bread, icings and glazes, Bohemian water rolls, black walnut bread, raisin whole wheat bread, poppyseed horns, cheesebread, and challis, all using honey. Glabau (1944, 1945) described formulas for various breads, cakes, and cookies.

TABLE XXII

NUTRIENTS IN HONEY IN RELATION TO HUMAN REQUIREMENTS[a]

Nutrient	Unit	Average amount in 100 gm honey	Recommended daily intake U.S.A.
Energy equivalent	kcal	304	2800
Vitamins:			
A	i.u.		5000
B_1 (Thiamin)	mg	0.004–0.006	1.5
B_2 complex:			
Riboflavin	mg	0.02–0.06	1.7
Nicotinic acid (niacin)	mg equiv.	0.11–0.36	20
B_6 (Pyridoxine)	mg	0.008–0.32	2.0
Pantothenic acid	mg	0.02–0.11	10
Folic acid	mg		0.4
B_{12}	μg		6.0
C (Ascorbic acid)	mg	2.2–2.4	60
D	i.u.		400
E	i.u.		30
H (Biotin)	mg		0.3
Minerals:			
Calcium	gm	0.004–0.03	1.0
Chlorine	mg	0.002–0.02	
Copper	mg	0.01–0.1	2.0
Iodine	mg		0.15
Iron	mg	0.1–3.4	18
Magnesium	mg	0.7–13	400
Manganese	mg	0.02–10	
Phosphorus	gm	0.002–0.06	1.0
Potassium	gm	0.01–0.47	
Sodium	gm	0.0006–0.04	
Zinc	mg	0.2–0.5	15

[a] Taken, with omissions, from Crane (1975a, p. 264).

Uses of honey in commercial baking have been rather thoroughly explored in a series of papers from Kansas State University. Advantages for honey-sweetened baked goods in moisture retention, texture, keeping quality, flavor, and the undefinable "eating quality" have been shown for white and whole wheat bread (Smith and Johnson, 1951), cakes and sweet doughs (Smith and Johnson, 1952), cake, cookie, and sweet goods production (Johnson and Smith, 1953), cookies (Smith and Johnson, 1953a), and fruit cakes (Smith and Johnson, 1953b). Further publications include a bulletin with commercial-scale recipes (Miller *et al.*, 1960) and home recipes (Johnson *et al.*, 1959) for the same products.

The recent popularity of the granola-type of breakfast cereal has provided an additional use for honey. Although the original granola recipe requires a considerable proportion of honey, formula modification and price competition has eliminated it or reduced it in some instances to only a token—literally "below the salt" in ingredient statements. Nevertheless, quality formulation and enlightened promotion should permit honey to retain a position in this area.

Certain confections have been and are always properly made with honey—the nougats, halvah, torrone—but, in general, as in other areas, honey has been largely displaced by less costly (and less flavorsome) sweeteners. The possibilities and instructions for using honey in confections have been described by Barth (1952), Anderson (1958, 1963), Meineke (1967), and Watson (1968).

Honey is an optimal sweetening ingredient in the FDA Standards of Identity for fruit butters, jellies, jams, and preserves, providing it either be the sole such ingredient or represent at least 20% of the solids in mixtures with certain other of the optional sweeteners. It is unlikely that any appreciable amount of honey is presently in such use.

The inclusion of 2–3% honey in prune juice is permitted under the standard of identity: a honey-sweetened canned grapefruit juice has enjoyed some recent success. Attempts to prepare beverages or beverage bases containing lemon juice and honey have been retarded by formation in storage of an unsightly floc. Recent experimentation (White, 1976) has shown that this can be eliminated by removing the colloidal materials in honey by treatment with bentonite (White and Walton, 1950).

A different type of fruit and honey product is a high-density honey-fruit spread. These spreads are easily made by mixing high-solids fruit juice concentrates with five to eight parts of full-density honey, followed by the controlled crystallization process described earlier. Grape, citrus, and berry flavors blend especially well with the honey flavor (White, 1950). Another process mixes comminuted dried fruits with honey, followed by controlled crystallization (Berthold and Benton, 1968).

Products containing honey intended for food manufacture include several dried mixtures. A spray-dried blend of 40% honey and 60% nonfat milk has been described (Walton *et al.*, 1951) and a tunnel-dried mix containing up to 70% honey was patented by Webb and Walton (1952). The milk solids content of these products limits their value to baking, beverage, or confectionery use. A continuous process using a wiped-film vacuum evaporator which can dry pure honey was described by Turkot *et al.* (1960); the product is highly hygroscopic, but suffers no flavor or color damage in the process. The addition of 35% sucrose before drying is recommended in order to improve storage capability by raising the softening point. A schematic diagram of the process is shown in Fig. 28. In spite of its hygroscopicity, limited testing has shown it not to induce caking in prepared dry baking mixes.

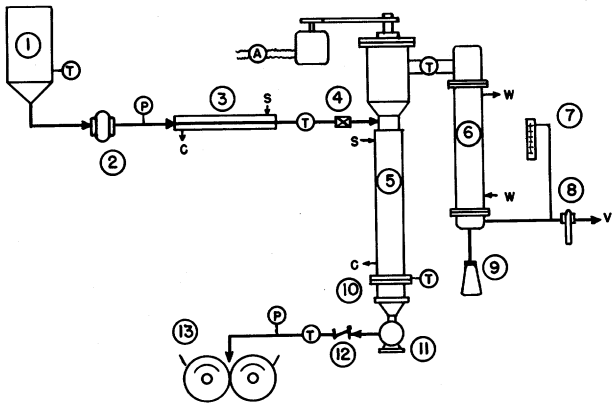


FIG. 28. Flowsheet and diagram of pilot-scale apparatus used to dehydrate honey. 1, feed tank; 2, feed pump; 3, feed preheater; 4, back pressure valve; 5, evaporator; 6, condenser; 7, absolute pressure manometer; 8, vacuum regulator; 9, condensate receiver; 10, sightglass; 11, product pump; 12, check valve; 13, chilling rolls. T, thermometer or thermocouple; S, steam; C, condensate; W, water; V, vacuum source; A, ammeter; P, pressure gauge. From Turkot *et al.*, 1960. Reprinted from Food Technology/*Food Technology* **14,** 387–390 (1960). Copyright © by Institute of Food Technologists.

Several dry honey mixtures are commercially available and produced under the patents of Straub (1954) in which gelatinized starch is used to aid drying, and Northcutt and Northcutt (1945) which covers atmospheric drum-drying.

In general, prospective users of honey in food products will seek either product or promotion advantages to offset the higher cost of the material. Its prime attribute is flavor, which cannot be discerned at very low ingredient levels but cannot be duplicated when honey is present at optimal amounts. Its nature as a somewhat acid reducing sugar or solution with distinctive flavor attributes must be kept in mind in formulation. Heat treatment to inactivate enzymes should be specified to insure product stability.

B. NONFOOD

Probably the largest nonfood use of honey is in pharmaceuticals. In addition to home use with lemon juice for easing sore throats, honey has been compounded into a number of successful commercial cough remedies. No effort will be made to review this area in view of the subject matter of the series; articles by Rubin *et al.* (1959) and Gennaro *et al.* (1959) may be consulted.

The use of honey in medicine is a subject reported intermittently for the past 4000 years. Since this is also outside the food area, the interested reader is referred to the review by Stomfay-Stitz (1960) for the earlier aspects; for more recent documented articles on the successful use of honey for wounds to Temnov

(1944), Gubin (1945), Bulman (1955); for infected wounds, to Gundel and Blattner (1934) and Zaiss (1934); for burns, to Phillips (1933), Voigtlander (1937); and to a remarkable article by Cavanagh *et al.* (1970) on the successful direct application to extensive wounds following surgery, in which undiluted honey-killed organisms cultured from the wounds of twelve patients. Willson and Crane (1975) have written an extensive review on honey uses.

IX. STANDARDS, SPECIFICATIONS, AND QUALITY CONTROL

A. UNITED STATES STANDARDS

There is no Federal Standard of Identity for honey; the general regulations for food products apply, of course. The definition from the earlier Act of 1906 is still considered a guide in what is considered honey: "Honey: The nectar and saccharine exudations of plants gathered, modified, and stored in the comb by honeybees (*Apis mellifera* and *apis dorsata*). Honey is levorotatory and contains not more than 25% of water, not more than 0.25% of ash, and not more than 8% of sucrose" (USFDA, 1936). This definition excludes honeydew honey or any honey containing honeydew sufficient to render it dextrorotatory. Criticism of the limits of moisture (too high), sucrose (too high), and ash (too low) have been expressed by White *et al.* (1962) and Feinberg (1951).

The U.S. Department of Agriculture has established voluntary grading standards for extracted honey and comb honey.* Honey is classified into seven color categories as indicated in Table XXIII. The USDA color comparator is a device in which honey in specified 2-oz square bottles is compared to glass standards representing the established limits of each grade (Brice *et al.*, 1956, 1965). Color is not a quality factor. Honey is assigned to one of four quality grades (U.S. Grade A or U.S. Fancy, U.S. Grade B or U.S. Choice, U.S. Grade C or U.S. Standard, and U.S. Grade D or Substandard) by evaluating solids content, flavor, absence of defects (particles of comb, propolis, or other material in suspension or deposited as sediment), and clarity. Moisture minima are 18.6% for the two upper grades, 20% for Grade C (honey for reprocessing), and unspecified for Grade D.

B. CODEX ALIMENTARIUS

The European Economic Community has directed the member countries to incorporate the FAO European Regional Standard for Honey (Codex Alimen-

*Copies of standards may be obtained from Chief, Processed Products Standardization and Inspection Branch, Fruit and Vegetable Division, Agricultural Marketing Service, USDA, Washington, DC 20250.

TABLE XXIII

STANDARD COLOR DESIGNATION OF HONEY AND RANGE FOR EACH COLOR[a]

USDA color standards	Color range USDA color standards	Color range Pfund scales (mm)	Optical density[b]
Water White	Honey that is Water White or lighter in color than Water White Color Standard.	8 or less	0.0945
Extra White	Honey that is darker than Water White but not darker than Extra White Color Standard.	Over 8 to and including 17	0.189
White	Honey that is darker than Extra White but not darker than White Color Standard.	Over 17 to and including 34	0.378
Extra Light Amber	Honey that is darker than White but not darker than Extra Light Amber or Golden Color Standard.	Over 34 to and including 50	0.595
Light Amber	Honey that is darker than Extra Light Amber but not darker than Light Amber Color Standard.	Over 50 to and including 85	1.389
Amber	Honey that is darker than Light Amber but not darker than Amber Color Standard.	Over 85 to and including 114	3.008
Dark Amber	Honey that is darker than Amber Color Standard.	Over 114	—

[a] Taken from USDA (1951).

[b] Optical density (absorbance) = $\log_{10}$ (100/percentage transmittance), at 560 nm for 3.15 cm thickness for caramel-glycerin solutions measured versus an equal cell containing glycerin.

tarius Commission, 1969) into their national honey legislation, with a few minor exceptions. The United States participated in the development of the standards but does not accept them.

In the standard, honey is defined as "the sweet substance produced by honey bees from the nectar of blossoms or from secretions of or on living parts of plants, which they collect, transform, combine with specific substances, and store in honey combs." Honey is classified according to origin as blossom or nectar honey and honeydew honey, and by processing mode as comb, extracted, or pressed honey. The compositional criteria are shown in Table XXIV. Other rules relate to flavor, absence of fermentation, extent of heat treatment, addition of acid, cleanliness, and labeling. Honey not meeting the criteria for diastase and HMF content or flavor, fermentation, and extent of heating must be sold as "baking honey" or "industrial honey," generally at lower prices. Methods of analyses are specified for the parameters listed in Table XXIV.

Information on and a comparison of grading rules and regulations for many countries are given in an article by Fasler (1975).

TABLE XXIV

CODEX ALIMENTARIUS: ESSENTIAL COMPOSITION AND QUALITY FACTORS FOR HONEY[a]

Compositional Criteria	
Apparent reducing sugar content, calculated as invert sugar:	
Blossom honey, when labeled as such:	not less than 65%
Honeydew Honey and blends of Honeydew Honey and Blossom Honey:	not less than 60%
Moisture content:	not more than 21%
Heather Honey (Calluna)	not more than 23%
Apparent sucrose content:	not more than 5%
Honeydew Honey, blends of Honeydew Honey and Blossom Honey, Robinia, Lavender and *Banksia menziesii* Honeys:	not more than 10%
Water-insoluble solids content:	not more than 0.1%
Pressed Honey:	not more than 0.5%
Mineral content (ash):	not more than 0.6%
Honeydew honey and blends of honeydew honey and blossom honey:	not more than 1.0%
Acidity:	not more than 40 meq/1000 gm
Diastase activity and hydroxymethylfurfural content:	
Determined after processing, blending; diastase figure on Gothe scale:	not less than 8
Provided the hydroxymethylfurfural content is:	not more than 40 mg/kg
Honeys with low natural enzyme content, *e.g.* citrus, diastase content on Gothe scale:	not less than 3
Provided the hydroxymethylfurfural content is:	not more than 15 mg/kg

[a] From Codex Alimentarius Commission (1969).

C. SPECIFICATIONS

Honey is generally traded by sample, but difficulties are sometimes encountered when either the sampling is not done in a representative fashion, or improper storage of the lot between sampling and delivery brings about an increase of color or possible fermentation. Variations can exist from drum to drum, or even in layers of a single drum, depending upon producers practices (Smith, 1967). Purchase from processors is generally not subject to these problems.

Honey purchased for food manufacturing use should be required to meet the appropriate U.S. Grade (A or B), and should be specified by color (Pfund or USDA) and, if appropriate, by floral blend, in general terms. Further requirements, if established for specific manufacturing use, should avoid unnecessary detail as to specific composition limits (other than moisture), since a needless burden is thus placed on the supplier for analytical services not ordinarily done.

An example of specification guidelines for purchase of honey for use in baking is given below (Smith and Johnson, 1951, 1952, 1953a,b; Johnson and Smith, 1953).

General

1. All honey containers should be clearly labeled, showing grade, floral source, moisture content, and color in mm Pfund, as well as U.S. Department of Agriculture color standards.
2. Honey for bakers' use should be "U.S. Grade A" or "B," according to U.S. standards for grades of extracted honey, effective April 16, 1951.
3. Honey should be heat-treated at 71°C for 30 minutes to retard granulation and enzyme activity.

White or Whole Wheat Bread

4. The Pfund colorimeter reading should not exceed 70 mm for honey to be used in white bread.
5. Predominant floral sources of Eastern buckwheat, fall flowers, heartsease, and tupelo honeys should not be used in white bread, except in blends as noted in item 7.
6. Eastern buckwheat, fall flowers, heartsease, and horsemint honeys should not be used in whole wheat bread, except in blends as noted in item 7.
7. Blends of acceptable honeys containing 10% of Eastern buckwheat, or 15% of heartsease, fall flowers, or tupelo honeys are acceptable.

Yeast-Leavened Sweet Goods

8. Predominant floral sources of Eastern buckwheat, fall flowers, heartsease, or horsemint honeys are not recommended for use in sweet goods, except in blends containing not more than 10% Eastern buckwheat, or 15% fall flowers, heartsease, or horsemint. Other honeys of acceptable flavor are satisfactory in yeast sweet goods.

Cake Products

9. Predominant floral sources of Eastern buckwheat, fall flowers, heartsease, and horsemint honeys are not desirable for use in white, yellow, or chocolate cakes.
10. Only honey classified as white by the U.S. grade and color standards is recommended for use in white cake.

Fruit Cake

11. Predominant floral sources of Eastern buckwheat and fall flowers honeys are not recommended for use in fruit cake.

12. Predominant floral sources of heartsease, Eastern buckwheat, tupelo, or eucalyptus are not recommended.

It is highly recommended, however, that only pressure-filtered honey be purchased for food manufacturing use; the USDA grade specification for defects could conceivably allow traces of undesirable material to be present.

D. QUALITY CONTROL

The parameters ordinarily monitored by food manufacturers will normally be moisture, flavor, color, and cleanliness. The refractometer is recommended for moisture, according to AOAC method 31.112 (Horwitz, 1975).

Honey color is generally evaluated in the honey industry by the Pfund Honey Color Grader, since it provides a continuous scale of color, useful in blending and assigning prices to bulk honey. The instrument is available from beekeeping supply houses. The less expensive (and easier to use) USDA honey color classifier can be used to assign a sample to its color class.

The use of a commercially available photometer for color classification was proposed by Townsend (1969); he used white light in an instrument accepting 19-mm test tubes and obtained acceptable correlation between absorbance and Pfund readings. He also demonstrated the use of the instrument for blending honeys.

Flavor must ultimately be judged subjectively, though Merz (1963) has proposed that GLC examination of an ether extract provides a simple procedure for objective assessment of honey flavor. He found the HMF peak to dominate in assays of extracts of honeys of "satisfactory" flavor and to be but a minor constituent of those of organoleptically unsatisfactory flavor.

Cleanliness is specified in the USDA grades as "shall be at least as free from defects as honey that has been strained through a No. 80 sieve (Grade A)... No. 50 sieve (Grade B)... No. 18 sieve (Grade C) at not over 130°F (54.4°C)." For food use, as noted above, pressure filtered (i.e., not simply strained) honey should be specified. The Codex requires a maximum of 0.1% in water-insoluble solids for honey, determined by filtering a 20-gm sample diluted with 80°C water through a tared sintered glass crucible (pore size 15–40 microns) and washed sugar-free with 80°C water, drying and weighing. A filtered honey should have negligible residue by this test.

A need for determining other compositional factors such as sugars, acids, and ash is not foreseen for quality control; methods are available in the AOAC book of methods (Horwitz, 1975). For defense against possible substitution of other syrups, the resorcinol test (AOAC Method 31.138, 31.139) or determination of HMF (Winkler, 1955) for acid inverted syrups and the AOAC test for corn syrup

(Method 37.134-6) are recommended. Samples with HMF values over 20 mg HMF/100 gm must be suspected unless a history of high-temperature storage can be proven.

A definitive test for the adulteration of honey with corn sirups, including the new high-fructose corn syrup has been developed (White and Doner, 1978). It is based on the difference in the ratio of ^{13}C to ^{12}C in the sample. Corn syrups are slightly enriched in ^{13}C in comparison with honey; variability in the ratio for honey is the lowest yet found for any honey constituent or property.

Since diastase is the more heat-resistant honey enzyme (Fig. 14), assay for diastase may be used when intended use of honey requires that enzyme activity be eliminated. Honey intended for export may be assayed for diastase to provide assurance of meeting Codex standards. Edwards *et al.* (1975) have compared a procedure using a proprietary chromogenic substrate (Amylochrome) with the Codex method. Agreement was excellent over the entire range and major savings in operator time resulted also. Use of this or a similar product should be considered for routine diastase measurement in honey.

X. RESEARCH NEEDS

Honey has been an article of commerce for many thousands of years and an object of research for perhaps one hundred. Much of the literature is still only descriptive, reporting values and variability in composition. A large proportion is devoted to detection of falsification or quality deterioration, but in recent years some understanding of the chemistry and biochemistry of the product is beginning to emerge. Because of the great complexity of honey, advances in understanding must often await improvements in analytical methods. "Understanding" based on inadequate analytical procedures must be reviewed and corrected when the opportunity arises. Generalizations are often based on inadequate numbers of samples tested because of limitations of time or funding; these must be placed upon a wider data base.

Current and anticipated developments in manufacture of corn and other syrups offer opportunity for falsification of honey that are increasingly difficult to detect. While current studies in this field may provide definitive methods, each improvement in commercial syrups must be examined in this light in order to maintain the integrity of the honey market. A few specific research needs are outlined below.

1. A broader base for prediction of enzyme stability and HMF accumulation in full-density honey upon storage and heating.

2. Assays of true glucose oxidase activities of honey, after removal by dialysis (or other means) of materials reactive to the hydrogen peroxide pro-

duced, in relation to peroxide accumulation values which are determined with diluted whole honey.

3. Development of an accurate method for gluconolactone and gluconic acid in honey.

4. Determination of the heat capacity of honey in the temperature range of processing interest and verification of published data on heat transfer coefficients.

5. Extension of the objective color classification of honey to photometers using a 1-cm cell to increase availability of equipment usable for this purpose.

6. Development of uses for honey in the food industry where its attributes cannot be duplicated by other syrups so as to broaden industrial use of honey, seriously eroded by less expensive refined sweetening agents.

Developments to maintain and strengthen markets for domestic honey can have an impact greatly exceeding the value of the honey itself. A strong honey market is of the greatest national importance because honey provides a large fraction of the beekeeper's income; beekeeping is vital to pollination of billions of dollars worth of food, feed, and fiber crops in the United States.

REFERENCES

Albanese, A. A., Higgons, R. A., Belmont, A., and DiLallo, R. 1954. Effect of age on the utilization of various carbohydrates by man. *Metab., Clin. Exp.* **3,** 154–159.

Albanese, A. A., Lorenze, E. J., and Orto, L. A. 1968. Effect of strokes on carbohydrate tolerance. *Geriatrics* **23,** 142–150.

Anderson, E. J. 1958. Honey candies. *Pa. Agric. Exp. Stn., Prog. Rep.* **186.**

Anderson, E. J. 1963. Maple-honey candies. *Glean. Bee Cult.* **91**(4), 230–234.

Aoyagi, S., and Oryu, C. 1968. Honeybees and honey. III. Yeasts in honey. *Tamagawa Daigaku Nogakubu Kenkyu Hokoku* **7/8,** 203–213.

Austin, G. H. 1958. Maltose content of Canadian honeys and its probably effects on crystallization. *Proc. Int. Congr. Entomol., 10th, Montreal, 1956* **4,** 1001–1006.

Barth, W. 1952. Report on the use of honey as an ingredient in confectionery. M.S. Thesis, Cornell Univ., Ithaca, New York.

Bates, F. J., and associates. 1942. Polarimetry, saccharimetry, and the sugars. *Natl. Bur. Stand. (U.S.), Circ.* **C440.**

Bauer, F. W. 1960. Honey marketing. *Calif., Agric. Exp. Stn., Bull.* **776,** 1–71.

Beck, B. F. 1938. "Honey and Health." McBride, New York.

Bergel, C., and Stuwe, G. G. 1972. Thermische Stoffbehandlung: Betrachtungen zur Honigerwärmung. *Suesswaren* **3,** 77–83.

Bergner, K. G., and Diemair, S. 1975. Proteins des Bienenhonigs. II Gelchromatographie, enzymatische Aktivität und Herkunft. *Z. Lebensm.-Unter. -Forsch.* **157,** 7–13.

Bergner, K. G., and Hahn, H. 1972. Zum Vorkommen und zur Herkunft der freien Aminosäuren in Honig. *Apidologie* **3**(1), 5–34.

Berthold, R., Jr., and Benton, A. W. 1968. Creamed honey-fruit spreads. *Food Techol.* **22**(1), 83–85.

Biino, L. 1971. Ricerca di alcuni aminoacidi in due varietà di miele. *Riv. Ital. Essenze Profumi* **53**(2), 80–84.

Borus, L., Kalinowski, J., and Zalewski, W. 1966. [Production and chemical composition of locust honey from the vicinity of Cigacice.] *Pszczelnicze Zesz. Nauk.* **10**(1/4), 113–122.

Braunsdorf, K. 1932. Zuckerfütterungshonig and Diastaseherkunft. *Z. Unter. Lebensm.* **64,** 555–558.

Brice, B. A., Turner, A., Jr., and White, J. W., Jr. 1956. Glass color standards for extracted honey. *J. Assoc. Off. Agric. Chem.* **39**(4), 919–937.

Brice, B. A., Turner, A., Jr., White, J. W., Jr., Southerland, F. L., Fenn, L. S., and Bostwick, E. P. 1965. Permanent glass color standards for extracted honey. *U.S. Dep. Agric., Agric. Res. Serv.* **AIC 73-48,** 1–6.

Browne, C. A. 1908. Chemical analysis and composition of American Honeys. *U.S. Dep. Agric., Bur. Chem. Bull.* **110,** 1–93.

Bulman, M. W. 1955. Honey as a surgical dressing. *Br. Bee J.* **83,** 664–665.

Burgett, D. W. 1974. Glucose oxidase: A food protective mechanism in social hymenoptera. *Ann. Entomol. Soc. Am.* **67**(4), 545–546.

Cale, G. H., Banker, R., and Powers, J. 1975. Management for honey production. *In* "The Hive and The Honeybee" (R. A. Grout, ed.), pp. 355–412. Dadant and Sons, Hamilton, Illinois.

Cavanagh, D., Beazley, J., and Ostapowicz, F. 1970. Radical operation for carcinoma of the vulva. *J. Obstet. Gynecol. Br. Commonw.* **77**(11), 1037–1040.

Chataway, H. D. 1932. Determination of moisture in honey. *Can. J. Res.* **6,** 532–547.

Chataway, H. D. 1933. The determination of moisture in honey by the hydrometer method. *Can. J. Res.* **8,** 435–439.

Cocker, L. 1951. The enzymic production of acid in honey. *J. Sci. Food Agric.* **2**(9), 411–414.

Codex Alimentarius Commission. 1969. "Recommended European Standard for Honey," CAC/RS-12-1969. Jt. FAO/WHO Food Stand. Program, Rome. Reprinted in *Bee World* **51**(2), 79–91 (1970).

Codounis, M. I. 1962. ["The Crystallization of Honey."] Min. Agric., Athens.

Conrad, E. C., and Palmer, J. K. 1976. Rapid analysis of carbohydrates by high-pressure liquid chromatography. *Food Technol.* **30**(10), 84, 86, 88–92.

Crane, E., ed. 1975a. "Honey: A Comprehensive Survey," 608 pp. Heinemann, London.

Crane, E. 1975b. The World's Honey Production, *In* "Honey: A Comprehensive Survey" (E. Crane, ed.), pp. 141–153. Heinemann, London.

Crane, E. 1975c. History of honey. *In* "Honey: A Comprehensive Survey" (E. Crane, ed.), pp. 439–488. Heinemann, London.

Cremer, E., and Riedmann, M. 1964. Identifizierung von gaschromatographisch getrennten Aromastoffen in Honigen. *Z. Naturforsch. B* **19,** 76–77.

Curti, R., and Riganti, V. 1966. Ricerche sugli aminoacidi del miele. *Rass. Chim.* **18**(6), 278–282.

Davies, A. M. C. 1975. Amino acid analysis of honeys from eleven countries. *J. Apic. Res.* **14**(1), 29–39.

Davies, A. M. C. 1976. The application of amino acid analysis to the determination of the geographic origin of honey. *J. Food Technol.* **11,** 515–523.

Dean, G. R. 1974. An unstable crystalline phase in the D-glucose-water system. *Carbohydr. Res.* **34,** 315–322.

De Boer, H. W. 1934. De invloed van den ouderdom op de samenstelling van honig. *Chem. Weekbl.* **31,** 482–487.

Deodikar, G. B., Thakar, C. V., Phadke, R. P., and Shah, N. P. 1957. Thixotropy in honey of *Carvia callosa*. *Indian Bee J.* **19,** 71–72.

Detroy, B. F. 1966. Determining film coefficient for a viscous liquid. *Trans. ASAE* **9**(1), 91–2, 93, 97.

Dittmar, J. H. 1935. Hygroscopicity of sugars and sugar mixtures. *Ind. Eng. Chem.* **27**(3), 333–335.

Dold, H., Du, D. H., and Dziao, S. T. 1937. Nachweis antibakterieller, hitze- und lichtempfindlicher Hemmungstoffe (Inhibine) im Naturhonig (Blütenhonig). *Z. Hyg. Infektionskr.* **120,** 155–167.

Doner, L. W. 1977. The sugars of honey—a review. *J. Sci. Food Agr.* **28,** 443–456.

Dörrscheidt, W., and Friedrich, K. 1962. Trennung von aromastoffen des Honigs mit Hilfe der Gas-Chromatographie. *J. Chromatogr.* **7**(1), 13–18.

Duisberg, H., and Gebelein, H. 1958. Über die Kontrolle von Erhitzungsschäden bei Honigen. *Z. Lebensm.-Unters. -Forsch.* **107**(6), 489–501.

Duisberg, H., and Hadorn, H. 1966. Welche Anforderungen sind an Handelshonige zu stellen? Vorschläge auf Grund der Statistischen Auswertung von ca 1600 Honig-Analysen. *Mitt. Geb. Lebensmittelunters. Hyg.* **57**(5), 386–407.

Duisberg, H., and Warnecke, B. 1959. Erhitzungs- und Lichteinfluss auf Fermente und Inhibine des Honigs. *Z. Lebensm.-Unters. -Forsch.* **111,** 111–119.

Dustmann, J. H. 1971a. Messung von Wasserstoffperoxid und Enzymaktivitat in mitteleuropäischen Honigen. *Z. Bienenforsch.* **9**(2), 66–73.

Dustmann, J. H. 1971b. Über du Katalaseactivität in Bienenhonig auf der Tracht der Heidekrautgewäschse (Ericaceae). *Z. Lebensm.-Unters. -Forsch.* **145,** 292–295.

Dustmann, J. H. 1972. Einfluss der Dialyse bei der Bestimmung der Saccharase-Aktivität in Honig. *Lebensm.-Wiss. Technol.* **5**(2), 70–71.

Dyce, E. J. 1935. Honey process and product. U.S. Patent No. 1,987,893.

Dzialoszýnski, L., and Kuik, K. 1963. Aktywnosc kwasnej fosfatazy α-amylazy i katalazy w miodach z okzolic torunia. *Pszczelnicze Zesz. Nauk.* **7**(1), 33–39.

Echigo, T., and Takenaka, T. 1973. Changes in erlose contents by honeybee invertase. *Nippon Nogei Kaguku Kaishi* **47**(3), 177–183.

Edwards, R. A., Faraji-Haremi, R., and Wootton, M. 1975. A rapid method for determining the diastase activity in honey. *J. Apic. Res.* **14**(1), 47–50.

Elser, E. 1924. Beiträge zur quantitativen Honiguntersuchung. *Arch. Bienenkd.* **6,** 118.

Erlenmeyer and Planta. 1874. Über die Fermente in den Bienen, im Bienenbrot, u. im Pollen und über einige Bestandteile des Honigs. *Chem. Zentralbl.* p. 790.

Fabian, F. W., and Quinet, R. I. 1928. A study of the cause of honey fermentation. *Mich., Agric. Exp. Stn. Tech. Bull.* **62,** 1–41.

Farnsteiner, K. 1908. Der ameisengesäuregehalt des Honigs. *Z. Unters. Nahr.- Genussm. Gebrauchsgegenstaende* **15,** 598–604.

Fasler, A. 1975. Honey Standards Legislation. *In* "Honey a Comprehensive Survey" (E. Crane, ed.), pp. 329–354. Heinemann, London.

Feinberg, B. 1951. Ash in honey. *Am. Bee J.* **91,** 471.

Fiehe, J. 1932. Über die Herkunft der Honigdiastase. *Z. Unters. Lebensm.* **63,** 329–331.

Gauhe, A. 1941. Über ein glukoseoxydierendes Enzym in der Pharynxdrüse der Honigbiene. *Z. Vgl. Physiol.* **28**(3), 211–253.

Gautier, J.-A., Renault, J., and Julia-Alvarez, M. 1961. Recherche du sucre interverti dans le miel. Première partie: critique des reactions de Fiehe et de Feder. *Annis. Falsif. Fraudes* **54,** 177–193.

Geddes, J. P. 1964. Packs honey in quantity. *Food Eng.* **36**(11), 100–101.

Gennaro, A. R., Sideri, C. N., Rubin, N., and Osol, A. 1959. Use of honey in medicinal preparations. *Am. Bee J.* **99,** 492–493.

Gillette, C. C. 1931. Honey catalase. *J. Econ. Entomol.* **24,** 605–606.

Giri, K. V. 1938. The chemical composition and enzyme content of Indian honey. *Madras Agric. J.* **26,** 68–72.

Glaubau, C. A. 1944. Honey—its use in cakes and cookies for the holiday season. *Bakers Wkly.* Oct. 16, pp. 40–42; Oct. 23, pp. 44–46; Oct. 30, pp. 56–58, 66; Nov. 6, pp. 48–50; Nov. 13, pp. 56–58, 69.

Glabau, C. A. 1945. How the various kinds of honey breads are made. Parts 1-5. *Bakers Wkly.* July 2, pp. 52–53; July 9, pp. 50–51; July 16, pp. 44–45; July 23, pp. 50–51; July 30, pp. 34–35.

Goldschmidt, S., and Burkert, H. 1955. Die Hydrolyse des cholinergischen Honigwirkstoffes und anderer Cholinester mittels Cholinesterasen und deren Hemmung im Honig. *Hoppe-Seyler's Z. Physiol. Chem.* **301,** 78–89.

Gonnet, M., and Lavie, P. 1963. Study of the adhesiveness of crystallized honey to the walls of glass vessels. *Abstr., Int. Beekeep. Congr., 19th, Prague* Pap. No. 38.

Gontarski, H. 1948. Ein Vitamin C oxydierendes Ferment der Honigbiene. *Z. Naturforsch. B* **3,** 245–249.

Gontarski, H. 1954. Fermentbiologische Studien an Bienen. I. Das physikochemische Verhalten der kolenhydratspaltenden Fermente. (A) invertierende Enzyme. *Verh. Dtsch. Ges. Angew. Entomol.* **12,** 186–197.

Gothe, F. 1914. Die Fermente des Honigs. *Z. Unters. Nahr.- Genussm. Gebrauchsgegenstaende* **28,** 273–286.

Green, G. W. 1951. The granulation of honey and its relation to the laws of crystallization. *Int. Beekeep. Congr., 14th, Leamington, Engl.* Pap. No. 18.

Greenleaf, C. A., and Browne, C. A. 1929. Some observations on the Fiehe test. *J. Assoc. Off. Agric. Chem.* **12,** 319–323.

Griebel, C. 1938. Vitamin C enthaltenden Honig. *Z. Unters. Lebensm.* **75,** 417–420.

Griffith, J. H. 1934a. Honey can be used in practically all baked goods. *Bakers Helper,* July 28, pp. 112–114, Aug. 11, 189–191.

Griffith, J. H. 1934b. Rolls made with honey. *Bakers Helper,* pp. 470, 474.

Griffith, J. H. 1934c. Varieties of bread made with honey. *Bakers Helper,* Dec. 1, pp. 876–877, 910.

Gubin, A. F. 1945. [The Beekeeping Institute during the war: Honey in medicine.] *Pschelovodstvo* **1,** 25–29.

Günther, F., and Burckhart, O. 1967. Bestimmung der sauren Gesamtphosphatase in Honig. *Dtsch. Lebensm.-Rundsch.* **63**(2), 41–44.

Guilbault, J. 1965. Crystallization of honey. Thesis. Ontario Agric. Coll., Guelph.

Gundel, M., and Blattner, V. 1934. Über die Wirkung des Honigs auf Bakterien und infizierte Wunden. *Arch. Hyg. Bakteriol.* **112,** 319–322.

Hadorn, H. 1964. Enthalten Orangenblüten und Lavendelblüten honige enzymhemmende Stoffe? *Ann. Abeille* **7**(4), 311–320.

Hadorn, H., and Kovacs, A. S. 1960. Zur Untersuchung und Beurteilung von auslandischem Bienenhonig auf Grund des Hydroxymethyl furfurol und Diastase gehaltes. *Mitt. Geb. Lebensmittelunters. Hyg.* **51,** 373–390.

Hadorn, H., and Zürcher, K. 1962. Zur Bestimmung der Saccharase-Aktivität in Honig. *Mitt. Geb. Lebensmittelunters. Hyg.* **53,** 6–28.

Hadorn, H., and Zürcher, K. 1963. Über Zuckerfutterungshonig. *Mitt. Geb. Lebensmittelunters. Hyg.* **54,** 322–330.

Hadorn, H., and Zürcher, K. 1974. Zuckerspektrum und Kristallisationstendenz von Honigen. *Mitt. Geb. Lebensmittelunters. Hyg.* **65,** 407–420.

Hadorn, H., Zürcher, K., and Doevelaar, F. H. 1962. Über Wärme- und Lagerschädigunsen von Bienenhonig. *Mitt. Geb. Lebensmittelunters. Hyg.* **53**(3), 191–229.

Hahn, H. 1970. Zum Gehalt und zur Herkunft der freien Aminosäuren in Honig. Dissertation, Univ. Stuttgart, Stuttgart.

Haydak, M. H. 1936. A prolonged test of milk and honey diet. *Minn. Med. J.* **19,** 774–776.

Haydak, M. H. 1955. The nutritional value of honey. *Am. Bee J.* **95**(5), 185–191.

Haydak, M. H., Palmer, L. S., Tanquary, M. C., and Vivino, A. E. 1942. Vitamin content of honeys. *J. Nutr.* **23,** 581–588.

Haydak, M. H., Palmer, L. S., Tanquary, M. C., and Vivino, A. E. 1943. The effect of commercial clarification on the vitamin content of honey. *J. Nutr.* **26,**(3), 319–321.

Haydak, M. H., Vivino, A. E., Boehrer, J. J., Bjorndahl, O., and Palmer, L. S. 1944. A clinical and biochemical study of cow's milk and honey as an essentially exclusive diet for adult humans. *Am. J. Med. Sci.* **207,** 209–219.

Heiduschka, A., and Kaufmann, G. 1913. Über die Saüeren im Honig. *Sueddtsch. Apoth.-Ztg.* **53,** 118–119.

Helvey, T. C. 1953. Colloidal constituents of dark buckwheat honey. *Food Res.* **18**(2), 197–205.

Helvey, T. C. 1954. Study on some physical properties of honey. *Food Res.* **19**(3), 282–292.

Hilger, A. 1904. Zur Kenntnis der im rechtsdrehend Koniferhonig vorkommenden Dextrine. *Z. Unters. Nahr.-Genussm. Gebrauchsgegenstaende* **8,** 110–126.

Horwitz, W., ed. 1975. "Official Methods of Analysis of the Association of Official Analytical Chemists." Assoc. Off. Anal. Chem., Washington, D.C.

Huber, R. E. 1975. The purification and study of a honey bee abdominal sucrase exhibiting unusual solubility and kinetic properties. *Arch. Biochem. Biophys.* **168,** 198–209.

Huber, R. E., and Mathison, R. D. 1976. Physical, chemical, and enzymatic studies on the major sucrase of honey bees (*Apis mellifera*). *Can. J. Biochem.* **54,** 153–164.

Hudson, C. S., and Sherwood, S. F. 1920. The occurrence of melezitose in honey. *J. Am. Chem. Soc.* **42,** 116–125.

Hunter, A., and Downs, C. E. 1945. The inhibition of arginase by amino acids. *J. Biol. Chem.* **157,** 427–446.

Jackson, R. F., and Silsbee, C. G. 1924. Saturation relation in mixtures of sucrose, dextrose, and levulose. *U.S. Dep. Commer., Technol. Pap., Bur. Stand.* **18,** 277–304.

Jacobs, M. B. 1955. Flavoring with honey. *Am. Perfum. Essent. Oil Rev.* **66**(1), 46–47.

Johnson, J. A., and Smith, L. B. 1953. The use of honey in cake, cookie and sweet goods production. *Am. Baker* **21**(1), 21, 57.

Johnson, J. A., Miller, D., and White, J. W., Jr. 1959. Honey in your baking. *Kans. State Univ., Ext. Serv., Circ.* **281,** 2–23.

Kalimi, M. Y., and Sohonie, K. 1964. Mahabaleschwar honey. II. Effect of storage on carbohydrates, acidity, hydroxymethylfurfural, colour, and diastase content of honey. *J. Nutr. Diet.* **1**(4), 265–268.

Kalimi, M. Y., and Sohonie, K. 1965. Mahabaleshwar Honey. III. Vitamin contents (ascorbic acid, thiamine, riboflavin, and niacin) and effect of storage on these vitamins. *J. Nutr. Diet.* **2**(1), 9–11.

Keilin, D., and Hartree, E. F. 1948. Properties of glucose oxidase (Notatin). *Biochem. J.* **42,** 221–229.

Kelly, F. H. C. 1954. Phase equilibria in sugar solutions. IV. Ternary system of water-glucose-fructose. *J. Appl. Chem.* **4,** 409–411.

Kelly, F. H. C. 1955. Phase equilibria in sugar solutions. VIII. The quaternary system sucrose-fructose-glucose-water. *J. Appl. Chem.* **5,** 120–122.

Kelly, F. H. C. 1957. Personal communication.

Kerkvliet, J. D., and van der Putten, A. P. J. 1973. The diastase number of honey: A comparative study. *Z. Lebensm.-Unters. -Forsch.* **153,** 87–93.

Kiermeier, F., and Köberlein, W. 1954. Über die Hitzeinaktivierung von Enzymen im Honig. *Z. Lebensm.-Untersuch. -Forsch* **98,**(5), 329–347.

Kitzes, G., Schuette, H. A., and Elvehjem, C. A. 1943. The B vitamins in honey. *J. Nutr.* **26**(3), 241–250.

Knapp, F. W. 1967. Methyl anthranilate content of citrus and non-citrus honeys. *Int. Beekeep. Congr., 21st, Prelim. Sci. Meet., Summ. Pap* No. 34, p. 67.

Knott, M. E., Shukers, C. F., and Schlutz, F. W. 1941. The effect of honey upon calcium retention in infants. *J. Pediatr.* **19,** 485–494.

Komamine, A. 1960. Amino acids in honey. *Suom. Kemistil. B* **33,** 185–187.

Lackett, J. J., and Wilson, W. T. 1971. Liquefying crystallized honey with a microwave oven. *Am. Bee J.* **111**(8), 300–301.

Lampitt, L. H., Hughes, E. B., and Rooke, H. S. 1929. Furfural and diastase in heated honey. *Analyst* **54,** 381–395.

Langer, J. 1915. Das (serologisch fassbare) Eiweiss des Honigs stammt von der Biene (Langer) und nicht auf dem Blütenstaube (Küstenmacher). *Biochem. Z.* **69,** 141–144.

Laxa, O. 1923. Méthode nouvelle et simple pour le dosage des albuminoides dans le miel. *Ann. Falsif. Fraudes* **16,** 286–289.

Lochhead, A. G. 1933. Factors concerned with the fermentation of honey. *Zentralbl. Bakteriol. Parasitenkd., Infektionstr., Abt. 2* **88,** 296–302.

Lochhead, A. G., and Farrell, L. 1930a. Soil as a source of infection of honey by sugar-tolerant yeasts. *Can. J. Res.* **3**(1), 51–64.

Lochhead, A. G., and Farrell, L. 1930b. Effect of preservatives on fermentation by sugar-tolerant yeasts from honey. *Can. J. Res.* **3,** 95–103.

Lochhead, A. G., and Farrell, L. 1931a. Accessory food substances for osmophilic yeasts. I. A bioactivator in honey stimulating fermentation. *Can. J. Res.* **5,** 529–538.

Lochhead, A. G., and Farrell, L. 1931b. The types of osmophilic yeasts found in normal honey and their relation to fermentation. *Can. J. Res.* **5,** 665–672.

Lochhead, A. G., and Heron, D. A. 1929. Microbiological studies of Honey. I. Honey fermentation and its cause. II. Infection by sugar-tolerant yeasts. *Can., Dep. Agric., Bull.* **116,** 47 pp.

Lothrop, R. E. 1932. Specific test for orange honey. *Ind. Eng. Chem., Anal. Ed.* **4,** 395–396.

Lothrop, R. E. 1937. Retention of moisture in honey. *Am. Bee J.* **77,** 281, 290–294.

Lothrop, R. E. 1939. The composition of honey and its utilization—relation of composition and viscosity. *Am. Bee J.* **79,** 130–133.

Lothrop, R. E. 1943. Saturation relations in aqueous solutions of some sugar mixtures with special reference to higher concentrations. Ph.D. Thesis, George Washington Univ., Washington, D.C.

Lothrop, R. E., and Gertler, S. I. 1933. Determination of amino acids and related compounds in honey. *Ind. Eng. Chem., Anal. Ed.* **5**(2), 103–105.

Lothrop, R. E., and Paine, H. S. 1931. Diastatic activity of some American honeys. *Ind. Eng. Chem.* **23,** 71–74.

Lothrop, R. E., and Paine, H. S. 1934. A new method for processing honey. *Am. Bee. J.* **74**(12), 542–543.

Lund, R. 1909. Albuminate im Naturhonig und Kunsthonig. *Z. Unters. Nahr.-Genussm. Gebrauchsgegenstaende* **17,** 128–130.

Lund, R. 1910. Über die Untersuchung des Bienenhonigs unter spezieller Berücksichtigung der stickstoffhaltigen Bestanteile. *Mitt. Geb. Lebensmittelunters. Hyg.* **1,** 38–58.

MacDonald, J. E. 1963. Honey pumps. *Glean. Bee Cult.* **91**(2), 85–87.

McDonald, J. L. 1964. Further studies on honey crystallization. M.S. Thesis, Cornell Univ., Ithaca, New York.

Maeda, S., Mukai, A., Kosugi, N., and Okada, Y. 1962. [The flavor components of honey.] *J. Food Sci. Technol.* **9**(7), 270–274.

Martin, E. C. 1958. Some aspects of hygroscopic properties and fermentation of honey. *Bee World* **39**(7), 165–178.

Marvin, G. E. 1928. The occurrence and characteristics of certain yeasts found in fermented honey. *J. Econ. Entomol.* **21**(2), 363–370.

Marvin, G. E. 1930. Further observations on the deterioration and spoilage of honey in storage. *J. Econ. Entomol.* **23**(2), 431–438.

Marvin, G. E., Peterson, W. H., Fred, E. B., and Wilson, H. F. 1931. Some of the characteristics of yeasts found in fermenting honey. *J. Agric. Res.* **43**(2), 121–131.

Maurizio, A. 1961. Zuckerabbau unter der Einwirkung der invertierenden Fermente in Pharynxdrü-

sen und Mitteldarm der Honigbiene (*Apis mellifica* L). 3. Fermentwirkung während der Uberwinterung bei Bienen der Ligustica-Rasse. *Insectes Soc.* **8**(2), 125–175.

Meineke, E. A. 1967. Honey candies for the retail trade. *Am. Bee J.* **107**(12), 454–457.

Merz, J. H. 1963. The objective assessment of honey aroma by gas chromatography. *J. Apic. Res.* **2**(1), 55–61.

Michelotti, P., and Margheri, G. 1969. Studio sul contenuto in aminoacidi del miele. *Sci. Aliment.* **15**(7), 179–180.

Miller, D., White, J. W., Jr., and Johnson, J. A. 1960. Honey improves baked products. *Kans. Agric. Exp. Stn. Bull.* **411,** 5–22.

Milum, V. G. 1939. Why does honey discolor during processing and storage? *Am. Bee J.* **79**(9), 445–447.

Milum, V. G. 1948. Some factors affecting the color of honey. *J. Econ. Entomol.* **41**(3), 495–505.

Mizusawa, H., and Matsumuro, H. 1968. [Studies on chemical composition of honey. IV. Composition of free amino acids.] *Tamagawa Daigaku Nogakubu Kenkyu Hokoku* **7-8,** 194–196.

Money, R. W., and Born, R. 1951. Equilibrium humidity of sugar solutions. *J. Sci. Food Agric.* **2,** 180–185.

Munro, J. A. 1943. The viscosity and thixotropy of honey. *J. Econ. Entomol.* **36,** 769–777.

Nelson, E. K., and Mottern, H. H. 1931. Some organic acids in honey. *Ind. Eng. Chem.* **23**(3), 335.

Nelson, J. M., and Cohn, D. J. 1924. Invertase in honey. *J. Biol. Chem.* **62,** 193–224.

Nelson, J. M., and Sottery, C. T. 1924. Influence of glucose and fructose on the rate of hydrolysis of sucrose by invertase from honey. *J. Biol. Chem.* **62,** 139–147.

Northcutt, R. T., and Northcutt, R. T., Jr. 1945. Drying hygroscopic plastics. U.S. Patent No. 2,391,033.

Nussbaumer, T. 1910. Beitrag zur Kenntnis der Honiggärung nebst Notizen über die chemische Zusammensetzung des Honigs. *Z. Unters. Nahr.-Genussm. Gebrauchsgegenstaende* **20,** 272–277.

Oppen, F. C., and Schuette, H. A. 1939. Viscometric determination of moisture in honey. *Ind. Eng. Chem., Anal. Ed.* **11,** 130–133.

Paine, H. S., Gertler, S. I., and Lothrop, R. E. 1934. Colloidal constituents of honey. Influence on properties and commercial value. *Ind. Eng. Chem.* **26**(1), 73–81.

Papadakis, P. E. 1929. Further findings on invertase from honey. *J. Biol. Chem.* **83,** 561–568.

Pawan, G. L. S. 1973. Fructose. *In* "Molecular Structure and Function of Food Carbohydrate" (G. G. Birch and L. F. Green, eds.), pp. 65–80. Wiley (Halsted Press), New York.

Petrov, V. 1974. Quantitative determination of amino acids in some Australian honeys. *J. Apic. Res.* **13**(1), 61–66.

Phillips, C. E. 1933. Honey for burns. *Glean. Bee Cult.* **61**(5), 284.

Pigman, W. W., and Goepp, R. M., Jr. 1948. "Chemistry of the Carbohydrates," p. 434. Academic Press, New York.

Pryce-Jones, J. 1952. "Stringiness" in honey and in sugar syrup fed to bees. *Bee World* **33**(9), 147–150, 154–155.

Pryce-Jones, J. 1953. The rheology of honey. *In* "Foodstuffs, Their Plasticity, Fluidity, and Consistency" (G. W. Scott Blair, ed.), pp. 148–176. North-Holland Publ., Amsterdam.

Rahmanian, M., Kouhestani, A., Ghavifekr, H., Ter-sarkissian, N., Olszynamarzys, A., and Donoso, G. 1970. High ascorbic acid content in some Iranian honeys. Chemical and biological assays. *Nutr. Metab.* **12**(3), 131–135.

Ramsey, R. J., and Milum, V. G. 1933. The discoloration of honey. *Am. Bee J.* **73,** 305–306.

Richter, A. A. 1912. Über einen osmophilen Organismus, den Hefepilz *Zygosaccharomyces mellis acidi* sp. n. *Mycol. Zentralbl.* **1**(3/4), 67–76.

Rinaudo, M. T., Ponzetto, C., Vidano, C., and Marletto, F. 1973. The origin of honey saccharase. *Comp. Biochem. Physiol. B* **46,** 245–251.

Rubin, N., Gennaro, A. R., Sideri, C. N., and Osol, A. 1959. Honey as a vehicle for medicinal preparations. *Am. J. Pharm.* **131,** 246–254.

Schade, J. W., Marsh, G. L., and Eckert, J. E. 1958. Diastase activity and hydroxy-methyl-furfural in honey and their usefulness in detecting heat alteration. *Food Res.* **23,** 446–463.

Schepartz, A. I. 1965a. The glucose oxidase of honey. II. Stereochemical substrate specificity. *Biochim. Biophys. Acta* **96,** 334–336.

Schepartz, A. I. 1965b. The glucose oxidase of honey. III. Kinetics and stoichiometry of the reaction. *Biochim. Biophys. Acta* **99,** 161–164.

Schepartz, A. I. 1966a. The glucose oxidase of honey. IV. Some additional observations. *Biochim. Biophys. Acta* **118,** 637–640.

Schepartz, A. I. 1966b. Honey catalase: occurrence and some general properties. *J. Apic. Res.* **5**(3), 167–176.

Schepartz, A. I., and Subers, M. H. 1964. The glucose oxidase of honey. I. Purification and some general properties of the enzyme. *Biochim. Biophys. Acta,* **85,** 228–237.

Schepartz, A. I., and Subers, M. H. 1966a. Catalase in honey. *J. Apic. Res.* **5**(1), 37–43.

Schepartz, A. I., and Subers, M. H. 1966b. Observations on honey diastase. *J. Apic. Res.* **5**(1), 45–48.

Schönfeld, A. 1927. Jak a jak rychle vytvářeji včely med. *Vestn. Cesk. Akad. Zemed. Ved.* **3**(3), 228–231.

Schuette, H. A., and Baldwin, C. L., Jr. 1944. Amino acids and related compounds in honey. *Food Res.* **9,** 244–249.

Schuette, H. A., and Huenink, D. J. 1937. Mineral constituents of honey. II. Phosphorus, calcium, magnesium. *Food Res.* **2,** 529–538.

Schuette, H. A., and Remy, K. 1932. Degree of pigmentation and its probable relationship to the mineral constituents of honey. *J. Am. Chem. Soc.* **54,** 2909–2913.

Schuette, H. A., and Templin, V. 1930. Some observations on the application of the formal titration to honey. *J. Assoc. Off. Agric. Chem.* **13,** 136–142.

Schuette, H. A., and Triller, R. E. 1938. Mineral constituents of honey. III. Sulfur and chlorine. *Food Res.* **3**(5), 543–547.

Schuette, H. A., and Woessner, W. W. 1939. Mineral constituents of honey. IV. Sodium and potassium. *Food Res.* **4**(4), 349–353.

Shannon, F. L. 1916. Detection of artificial invert sugar in honey. *J. Assoc. Off. Agric. Chem.* **2,** 169–174.

Sherwood, J. 1924. Report on honey—detection of artificial invert sugar in honey. *J. Assoc. Off. Agric. Chem.* **7,** 345–349.

Siddiqui, I. R. 1970. The sugars of honey. *Adv. Carbohydr. Chem. Biochem.* **25,** 285–309.

Siddiqui, I. R., and Furgala, B. 1967. Isolation and characterization of oligosaccharides from honey. Part I. Disaccharides. *J. Apic. Res.* **6**(3), 139–145.

Siddiqui, I. R., and Furgala, B. 1968a. Isolation and characterization of oligosaccharides from honey. Part II. Trisaccharides. *J. Apic. Res.* **7**(1), 51–59.

Siddiqui, I. R., and Furgala, B. 1968b. Centose: A branched trisaccharide containing α-Δ-(1→2) and α-D-(1→4) glycoside bonds. *Carbohydr. Res.* **6,** 250–252.

Smith, F. G. 1965. The sucrose content of Western Australian honey. *J. Apic. Res.* **4**(3), 177–184.

Smith, F. G. 1967. Deterioration of the colour of honey. *J. Apic. Res.* **6**(2), 95–98.

Smith, L. B., and Johnson, J. A. 1951. The use of honey in bread products. *Bakers Dig.* **25**(6), 103–106.

Smith, L. B., and Johnson, J. A. 1952. The use of honey in cake and sweet doughs. *Bakers Dig.* **26**(6), 113–118.

Smith, L. B., and Johnson, J. A. 1953a. Honey—its value and use in popular cookies. *Bakers Dig.* **28**(2), 28–31.

Smith, L. B., and Johnson, J. A. 1953b. Honey improves fruit cake quality. *Bakers Dig.* **28**(3), 52–54.

Stinson, E. E., Subers, M. H., Petty, J., and White, J. W., Jr. 1960. The composition of honey. V. Separation and identification of the organic acids. *Arch. Biochem. Biophys.* **89,** 6–12.

Stomfay-Stitz, J. 1960. Honey; an ancient yet modern medicine. *Sci. Couns.* **23**(4), 110, 111, 116–118, 120–122, 125.

Straub, W. F. 1954. Solidified honey product and process for making same. U.S. Patent No. 2, 693, 420.

Subers, M. H., Schepartz, A. I., and Koob, R. P. 1966. Separation and identification of some organic phosphates in honey by column and paper chromatography. *J. Apic. Res.* **5**(1), 49–57.

Takenaka, T., and Echigo, T. 1975. Preliminary studies on purification of honey enzyme by DEAE-cellulose chromatography. *Tamagawa Daigaku Nogakubu Kenkyu Hokoku* **15,** 30–36.

Täufel, K., and Müller, K. 1953. Zur Herkunft der Saccharide im Honig. *Z. Lebensm.-Unters. -Forsch.* **96**(2), 81–83.

Täufel, K., and Müller, K. 1957. Analytische und Papierchromatographische Untersuchung über die Saccharide von Honig und Kunsthonig. *Ernaehrungsforschung* **2**(1), 70–82.

Temnov, V. A. 1944. Bacteriocidal properties of honey and utilization of honey and other beekeeping products for the healing of wounds. *Bee World* **25**(11), 86–87.

Ten Hoopen, H. J. G. 1963. Flüchtige Carbonylverbindungen in Honig. *Z. Lebensm.-Unters. -Forsch.* **119**(6), 478–482.

Thöni, J. 1913. Über Wesen und Bedeutung der quantitativen Präzipitinreaktion bei Honiguntersuchungen. *Z. Unters. Nahr.-Genussm. Gebrauchsgegenstaende* **25,** 490–493.

Thomson, R. H. K. 1938. Granulation problems in New Zealand Honey. *N.Z. J. Sci. Technol.* **20,** 714–720.

Tillmans, J., and Kiesgen, J. 1927. Die Formoltitration als Mittel zur Unterscheidung von künstlichen und natürlichen Lebensmitteln. *Z. Unters. Lebensm.* **53,** 131–137.

Tong, S. S. C., Morse, R. A., Bache, C. A., and Lisk, D. J. 1975. Elemental analysis of honey as an indicator of pollution. *Arch. Environ. Health* **30,** 329–332.

Townsend, G. F. 1954a. Honey—the energy food for athletes. *Am. Bee J.* **94**(7), 260–262.

Townsend, G. F. 1954b. Personal communication.

Townsend, G. F. 1969. Optical density as a means of colour classification of honey. *J. Apic. Res.* **8**(1), 29–36.

Townsend, G. F. 1975. Processing and storing liquid honey. *In* "Honey: A Comprehensive Survey" (E. Crane, ed.), pp. 269–292. Heinemann, London.

Turkot, V., Eskew, R. K., and Claffey, J. B. 1960. A continuous process for dehydrating honey. *Food Technol.* **14**(8), 387–390.

U.S. Department of Agriculture. 1951. "United States Standards for Grades of Extracted Honey," 4th Issue. Agric. Mark. Serv., Washington, D.C.

U.S. Department of Agriculture. 1975. "Honey," Foreign Agric. Circ. Foreign Agric. Serv., Washington, D.C.

U.S. Food and Drug Administration. 1936. "Definitions and Standards for Food Products for Use in Enforcing the Food and Drug Act," Serv. Regul. Announce., 5th Rev., No. 2. USFDA, Washington, D.C.

Vansell, G. H., and Freeborn, S. B. 1929. Preliminary report on the investigations of the source of diastase in honey. *J. Econ. Entomol.* **22,** 922–927.

Van Voorst, F. T. 1941. Biochemische suikerbepalingen. XII. Honing. *Chem. Weekbl.* **38,** 538–542.

Vignec, J., and Julia, J. F. 1954. Honey in infant feeding. *Am. J. Dis. Child.* **88,** 443–451.

Villumstad, E. 1952. Undersøkelser av behandlingens innflytelse på honningkvaliteten. *Aersmeld. St. Smaebrlaerarsk.* **13,** 1–25.

Voigtlander, H. 1937. Honey for burns and scalds. *Bee World* **18**(11), 128.

Von Fellenberg, T., and Rusiecki, W. 1938. Bestimmung der Trübung und der Farbe des Honigs.

Mitt. Geb. Lebensmittelunters. Hyg. **29,** 313–335.

Von Philipsborn, H. 1952. Über Calciumoxalat in Pflanzenzellen. *Protoplasma* **41**(4), 415–424.

Walton, G. P., White, J. W., Jr., Webb, B. H., Hufnagel, C. F., and Stevens, A. H. 1951. Manufacture of concentrated milk and honey products. *Food Technol.* **5**(5), 203–207.

Watanabe, T., and Aso, K. 1959. Isolation of kojibiose from honey. *Nature (London)* **183,** 1740.

Watson, C. D. 1968. A step-by-step guide to making fondant from pure honey. *Candy Ind. Confect. J.* **131**(13), 7–8.

Webb, B. H., and Walton, G. P. 1952. Dried honey-milk product. U.S. Patent No. 2,621,128.

Webb, J. L. 1963. "Enzyme and Metabolic Inhibitors," Vol. 1, pp. 59, 118, 160. Academic Press, New York.

Wedmore, E. B. 1955. The accurate determination of the water content of honeys. I. Introduction and results. *Bee World* **36**(11), 197–206.

Weishaar, H. 1933. Untersuchungen über Bestimmung, Mindeswert, und Herkunft der Honigdiastase. *Z. Unters. Lebensm.* **65,** 369–399.

White, J. W., Jr. 1950. New crystallized fruit spread shows commercial promise. *Food Ind.* **22,** 1216, 1298.

White, J. W., Jr. 1952. The action of invertase preparations. *Arch. Biochem. Biophys.* **39**(1), 238–240.

White, J. W., Jr. 1966. Methyl anthranilate content of citrus honey. *J. Food Sci.* **31,** 102–104.

White, J. W., Jr. 1967. Measuring honey quality—a rational approach. *Am. Bee J.* **107**(10), 374–375.

White, J. W., Jr. 1969. Moisture in honey: Review of chemical and physical methods. *J. Assoc. Off. Anal. Chem.* **52,** 729–737.

White, J. W., Jr. 1973. Toxic honeys. *In* "Toxicants Occurring Naturally in Foods," pp. 495–507. Comm. Food Prot., Natl. Acad. Sci., Washington, D.C.

White, J. W., Jr. 1975a. Composition of honey. *In* "Honey: A Comprehensive Survey" (E. Crane, ed.), pp. 157–206. Heinemann, London.

White, J. W., Jr. 1975b. Honey. *In* "The Hive and the Honeybee" (R. A. Grout, ed.), pp. 491–530. Dadant and Sons, Hamilton, Illinois.

White, J. W., Jr. 1976. Unpublished observations.

White, J. W., Jr., and Doner, L. W. 1978. Mass spectrometric detection of high fructose corn sirup in honey by $^{13}C/^{12}C$ ratio: collaborative study. *J. Assoc. Off. Anal. Chem.* **61,** 746–750.

White, J. W., Jr., and Hoban, N. 1959. Composition of honey. IV. Identification of the disaccharides. *Arch. Biochem. Biophys.* **80**(2), 386–392.

White, J. W., Jr., and Kushnir, I. 1966. Enzyme resolution in starch gel electrophoresis. *Anal. Biochem.* **16,** 302–313.

White, J. W., Jr., and Kushnir, I. 1967a. The enzymes of honey: Examination by ion-exchange chromatography, gel filtration, and starch-gel electrophoresis. *J. Apic. Res.* **6**(2), 69–89.

White, J. W., Jr., and Kushnir, I. 1967b. Composition of honey. VII. Proteins. *J. Apic. Res.* **6**(3), 163–178.

White, J. W., Jr., and Maher, J. 1953a. Transglucosidation by honey invertase. *Arch. Biochem. Biophys.* **42**(2), 360–367.

White, J. W., Jr., and Maher, J. 1953b. α-Maltosyl β-D-fructofuranoside, a trisaccharide enzymically synthesized from sucrose. *J. Am. Chem. Soc.* **75,** 1259–1260.

White, J. W., Jr., and Maher, J. 1954. Selective adsorption method for determination of the sugars of honey. *J. Assoc. Off. Agric. Chem.* **37**(2), 466–478.

White, J. W., Jr., and Subers, M. H. 1963. Studies on honey inhibine. 2. A chemical assay. *J. Apic. Res.* **2**(2), 93–100.

White, J. W., Jr., and Subers, M. H. 1964a. Studies on honey inhibine. 3. Effect of heat. *J. Apic. Res.* **3**(1), 45–50.

White, J. W., Jr., and Subers, M. H. 1964b. Studies on honey inhibine. 4. Destruction of the

peroxide accumulation system by light. *J. Food Sci.* **29**(6), 819–828.
White, J. W., Jr., and Walton, G. P. 1950. Flavor modification of low-grade honeys. *U.S. Dep. Agric., Bur. Agric. Ind. Chem.* **AIC-272,** 1–13.
White, J. W., Jr., Petty, J., and Hager, R. B. 1958. The composition of honey. II. Lactone content. *J. Assoc. Off. Agric. Chem.* **41**(1), 194–197.
White, J. W., Jr., Riethof, M. L., and Kushnir, I. 1961. Composition of honey. VI. The effect of storage on carbohydrates, acidity, and diastase content. *J. Food Sci.* **26**(1), 63–71.
White, J. W., Jr., Riethof, M. L., Subers, M. H., and Kushnir, I. 1962. Composition of American honeys. *U.S., Dep. Agric., Tech. Bull.* **1261,** 1–124.
White, J. W., Jr., Subers, M. H., and Kushnir, I. 1963a. How processing and storage affect honey quality. *Glean. Bee Cult.* **91,** 422–425.
White, J. W., Jr., Subers, M. H., and Schepartz, A. I. 1963b. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose oxidase system. *Biochim. Biophys. Acta* **73,** 57–70.
White, J. W., Jr., Kushnir, I., and Subers, M. H. 1964. Effect of storage and processing temperatures on honey quality. *Food Technol.* **18**(4), 153–156.
Willson, R. B. 1975. World trading in honey. *In* "Honey: A Comprehensive Survey" (E. Crane, ed.), pp. 355–377. Heinemann, London.
Willson, R. B., and Crane, E. 1975. Uses and products of honey. *In* "Honey: A Comprehensive Survey" (E. Crane, ed.), pp. 378–391. Heinemann, London.
Wilson, H. F., and Marvin, G. E. 1929. On the occurrence of yeasts which may cause the spoilage of honey. *J. Econ. Entomol.* **22,** 513–517.
Wilson, H. F., and Marvin, G. E. 1931. The effect of temperature on honey in storage. *J. Econ. Entomol.* **24,** 589–597.
Wilson, H. F., and Marvin, G. E. 1932. Relation of temperature to the deterioration of honey in storage. A progress report. *J. Econ. Entomol.* **25,** 525–528.
Winkler, O. 1955. Beitrag zum Nachweis und zur Bestimmung von Oxymethylfurfural in Honig und Kunsthonig. *Z. Lebensm.-Untersuch. -Forsch.* **102**(3), 161–167.
Wootton, M., Edwards, R. A., Faraji-Haremi, R., and Johnson, A. T. 1976a. Effect of accelerated storage conditions on the chemical composition and properties of Australian honeys. 1. Colour, acidity, and total nitrogen content. *J. Apic. Res.* **15**(1), 23–28.
Wootton, M., Edwards, R. A., and Faraji-Haremi, R. 1976b. Effect of accelerated storage conditions on the chemical composition and properties of Australian honeys. 2. Changes in sugar and free amino acid contents. *J. Apic. Res.* **15**(1), 29–34.
Zaiss. 1934. Der Honig in äusserlich Anwendung. *Muench. Med. Wochenschr.* **11,** 1891.
Zalewski, W. 1965. Fosfatazy w miodach. *Pszczelnicze Zesz. Nauk.* **9**(1-2), 1–34.